

SOME PHYSIOLOGICAL ASPECTS OF EVOLUTION IN WHEAT

by

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Except where acknowledged, work in this thesis is entirely
original.

R. L. Dunstone

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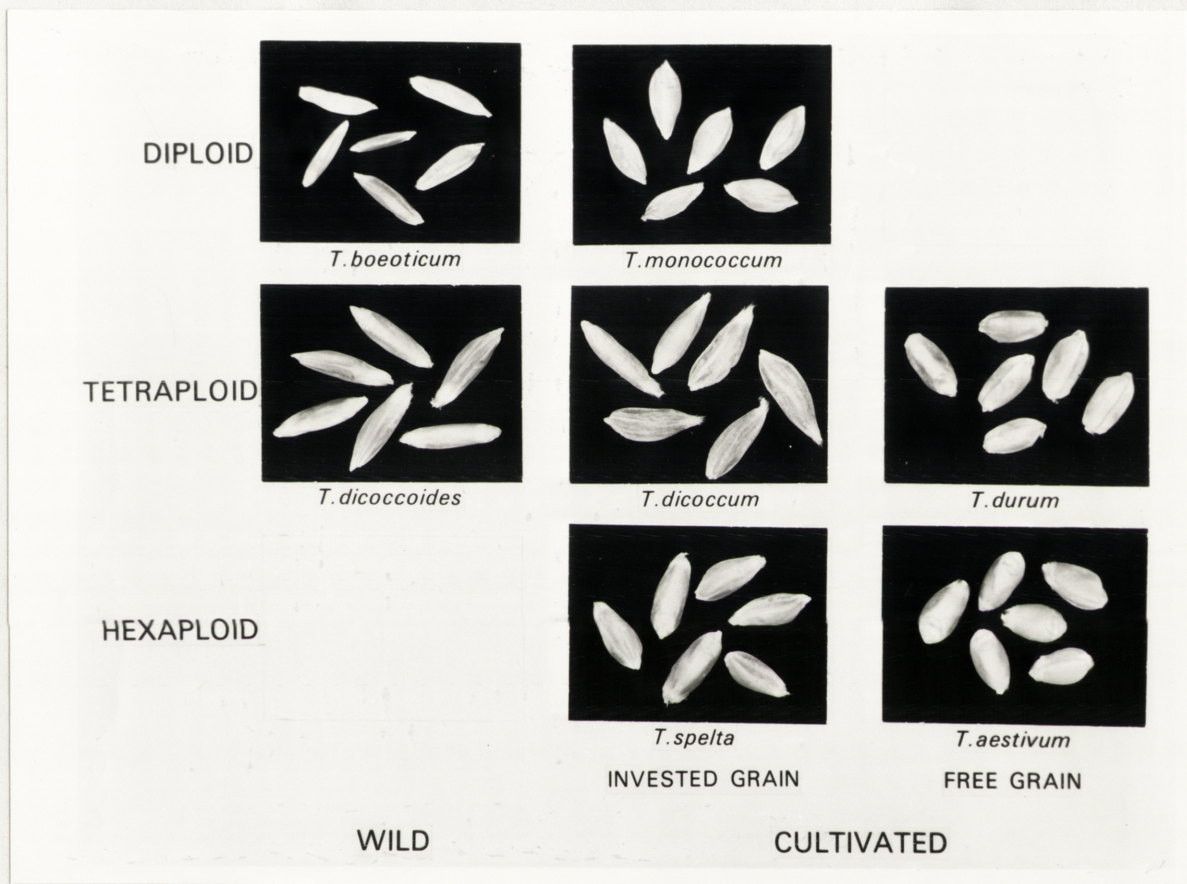
The project reported in this thesis was originally conceived by Dr. L. T. Evans. I wish to thank him for allowing me to take over the project, for working with me on the experimentation during Experiment 1, and for continued advice during the subsequent experiments.

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Frontispiece.

Selected grains of wheats arranged to show the changes which have taken place with increase in ploidy and with selection for cultivation. X1.5.

SUMMARY

Twenty three lines representing wild progenitors and cultivated wheats at the diploid, tetraploid and hexaploid levels were used in six experiments examining changes, during the evolution of wheat, in the capacity of processes that may limit grain yield.

Grain weight was found to increase with ploidy from the diploid to the tetraploid but not from the tetraploid to the hexaploid level. There was an increase in the grain weight with the shift from the wild to the cultivated species.

Paralleling the increase in grain weight with evolution there was an increase in leaf area. The parallel increase in grain and leaf size was not due to a parallel increase in cell size in both organs. Leaf area was positively related to the mean projected area of the mesophyll cells but there was no correlation between grain volume and endosperm cell cross-sectional area.

When the plants were grown under conditions of high light intensity there was a negative correlation between the area of the flag leaf blade and the photosynthetic rate per unit leaf area. Photosynthetic rate has therefore fallen in the course of evolution although total photosynthesis per flag leaf has increased due to the much larger leaves of more advanced lines.

The differences observed between the rates of photosynthesis of the early leaves of the various species were smaller than those between the flag leaves.

With flag leaves, the maximum rate of photosynthesis varied with the light intensity under which the plants were grown, but to markedly different extents in the various species. In the diploid Triticum species photosynthetic rate increased progressively

the higher the light intensity during growth. Species of Aegilops and tetraploid Triticum reached their maximum rates under moderate radiation levels. The photosynthetic rate of the hexaploid Triticum species was depressed when the plants were grown under high light conditions.

It could not be demonstrated that changes in stomatal frequency or specific leaf weight were consistently associated with changes in photosynthetic rate due to genotype, ontogenetic or radiation level during development.

Evolution in wheat appears to have been accompanied by increased apical dominance so that the total plant dry weight is concentrated in fewer tillers and, in addition, a greater part of the weight of each tiller is concentrated in the grain. A much higher proportion of the assimilates of the flag and the penultimate leaves is transported to the grain in the modern wheats.

Grain density has not changed during evolution, the increase in grain weight being associated with an increase in grain volume and a decrease in the percentage of nitrogen in the grain.

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INTRODUCTION

The present study was initiated to gain more insight into the factors controlling yield through an examination of the physiological changes that have accompanied the evolution of wheat.

Although much is known of the cytogenetics of the evolution of wheat, relatively little is known of the physiological changes that must have accompanied and contributed to this evolution. Vavilov (1951) suggested that an early step in examining "evolution directed by the will of man" was to establish the changes that have occurred during the evolution of the plant in the capacity or potential of the processes that may limit yield. Only recently has this suggestion been taken up and a start made on the study of such changes during evolution.

Schwanitz (1966, pages 15 and 16) emphasised the "gigantism" of cultivated plants. "Cultivated plants, as a rule differ from their wild forms by having larger and especially broader and thicker leaves; sturdier sprouts, stems and stalks; larger and fleshier roots and larger flowers, fructification, fruits and seeds". The increase in size with cultivation affects different parts of the plant in different ways, the fruits and seeds often increasing disproportionately to the rest of the plant. Large fruits and seeds are advantageous to man and are often found in cultivation.

Wheat is one of the few cultivated plants for which the wild progenitors and the main steps in its evolution are known with some confidence (Bell, 1965; Riley, 1965) and for which the progenitors are available in a wide range of forms. The main steps in the evolution of wheat are summarised in Figure 1 : 1:

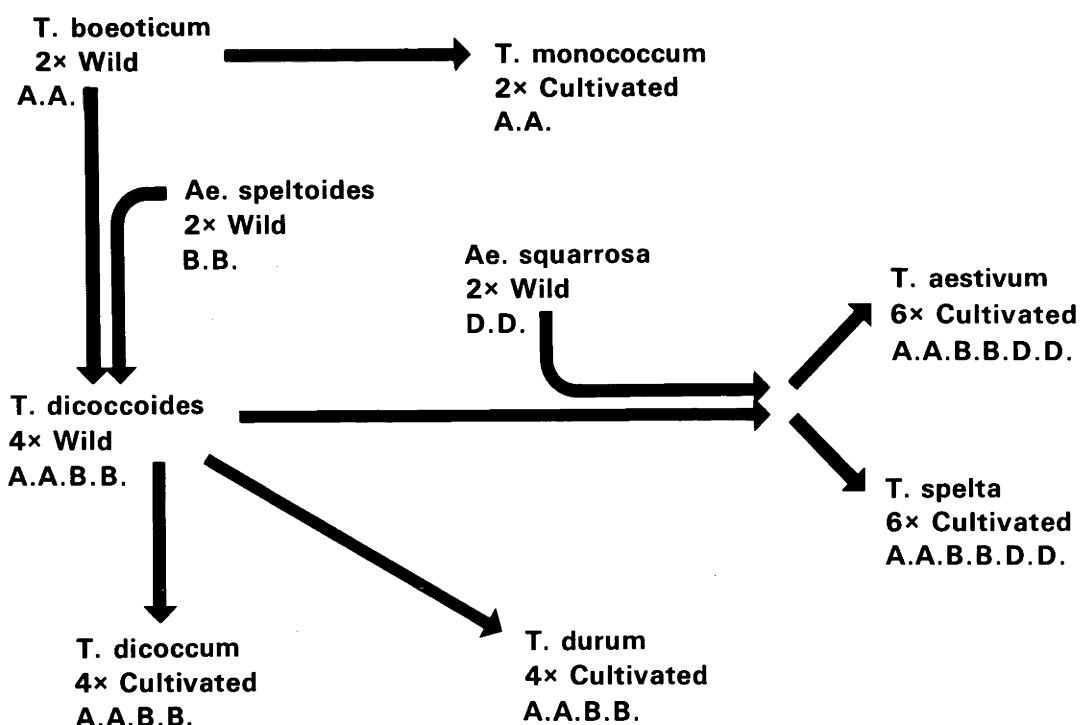
1. From wild diploid wheat, Triticum boeoticum, developed the cultivated einkorn, T. monococcum. Harlan and Zohary (1966) state that the most conspicuous difference between them is in the less fragile rachis of einkorn, the ear remaining intact until threshing.

2. Either T. boeoticum or T. monococcum hybridised with a species of Aegilops, probably Ae. speltoides or possibly Ae. bicornis, to give rise to the wild species T. dicoccoides.

3. T. dicoccoides gave rise, under primitive selection to emmer wheat, T. dicoccum, and

4. possibly by a single mutation, to the free threshing T. durum.

5. T. dicoccoides hybridised with Ae. squarrosa to give rise to the cultivated hexaploid wheats. The evolutionary relation between forms with invested grains such as T. spelta, and those with free threshing ears such as T. aestivum is not clear (Helbaek, 1960; Bell, 1965).



The progenitors of modern wheats are found in a wide variety of forms. Wild forms are found at two levels of ploidy (diploid and tetraploid) and cultivated forms at three levels of ploidy. The free availability of such material makes wheat an ideal plant for evolutionary studies.

Weight per grain has increased very substantially with evolution in wheat. Increases have taken place both with increase in ploidy and with selection at the diploid and tetraploid levels (Bell, Lupton and Riley, 1955).

An increase in cell size in wheat has been demonstrated both with selection from wild to cultivated at two levels of ploidy and with the increase in ploidy from diploid to tetraploid (Schwanitz, 1966, page 18), and these increases may well account for the increases observed in grain weights.

Paralleling the increase in grain size with evolution, there has been an increase in leaf area of the wheat plant. Growth studies by Kranz showed that the greater leaf area production per shoot in the larger grained polyploid species T. spelta and T. dicoccum than in the smaller grained diploid T. monococcum was associated with a more rapid rate of establishment and a longer period of growth of the polyploid species (Kranz, 1966b, Figure 1).

Selection at the tetraploid level has resulted in the cultivated T. dicoccum having a faster rate of leaf area increase than the wild T. dicoccoides, and attaining a greater final leaf area per plant. However, in T. durum, the most highly bred tetraploid species,

the rate of leaf area increase and the final leaf area were little greater than the corresponding values for its wild progenitor (Kranz, 1966b). This could be due to the low tillering rate after anthesis in highly bred wheats.

The maximum leaf area index (leaf area per unit ground area) of a crop increased twofold with selection for cultivation at both the diploid and tetraploid levels but did not increase with increase in ploidy (Kranz, 1966).

In all wheat species the leaf area ratio (leaf area per unit plant dry weight) decreased with increase in dry weight. The leaf area ratio decreased more rapidly with increase in plant dry weight in diploids generally and in one tetraploid species than with more highly evolved species (Khan and Tsunoda, 1970c).

Total dry weight of the plant was found to increase with ploidy from diploid to tetraploid (Khan and Tsunoda, 1970c). This result was not demonstrated by Kranz, but he did note a slight increase with selection at the tetraploid level (Kranz, 1966b, Figure 9).

The distribution of dry weight within the plant has changed with evolution. Kranz (1966b) noted a higher stem weight to root weight in the highly selected T. durum than the other tetraploids. Cultivated lines had a higher leaf dry weight to root weight ratio (Khan and Tsunoda, 1970c).

Kranz's data shows that the dry weight at maturity of all the ears of a plant increased dramatically from diploid to tetraploid lines, but decreased with selection at the tetraploid level (Kranz, 1966b, Figure 10). This apparent anomaly may well be due to the high number of tillers found in the wild species. Amongst cultivated wheats with invested grains, the total dry weight of ears was greatest in the hexaploid T. spelta, but this was only about 60% of the value in the wild T. dicoccoides.

The rate of dry weight increase of the ears has increased with selection at the tetraploid level, and also increased with the rise in ploidy from diploid to tetraploid but not from tetraploid to hexaploid for cultivated invested grain wheats (Kranz, 1966b, Figure 11).

Belikov, Motorina and Kurkova (1961) measured the photosynthetic rate of the second leaves of young wheat plants and found no differences in rate between T. monococcum, T. dicoccum and T. aestivum, in either low or high light. The maximum rates obtained were about $38 \text{ mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$ at approximately 10,000f.c. intensity.

A study of the photosynthetic potential of various Aegilops species showed maximum rates up to $35 \text{ mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$ (Naaber, 1964).

Thus when the experimental work for this thesis was begun, the results of previous investigations suggested that there had been little change, and certainly no rise in photosynthesis rate per unit leaf area during evolution in wheat.

Our early experiments indicated that photosynthesis rates were actually higher in the wild diploids than in the cultivated hexaploids and Khan and Tsunoda (1970a) recorded similar findings. In their material at each level of ploidy the unselected wild species had higher rates of photosynthesis than the cultivated lines. Winter lines had generally higher rates than the equivalent spring types. The higher rates of photosynthesis were associated with the small leaves of the wild diploids. The differences between the photosynthesis rates of the different lines was greatest at the highest light intensity used for measurement.

Correlations were found between the photosynthesis rate and nitrogen content per unit area, and also specific leaf weight (Khan and Tsunoda, 1970b). Photosynthesis rate also correlated with

transpiration rate, but as leaf temperatures were not quoted, the extent of stomatal control of photosynthesis cannot be inferred.

The ultimate productivity of a continuous crop stand must depend on its net photosynthesis rate per unit area and on the partitioning of assimilate into the grain. The work presented here was planned to examine the changes with evolution in a number of physiological systems which seemed likely to be important in the limitation of yield in wheat. The growth patterns of leaves and grains, and the distribution of dry weight throughout the various plant parts were first examined. In addition the distribution of ^{14}C labelled assimilates from fed flag leaves and penultimate leaves was traced.

As the photosynthetic rate seemed to be of primary importance to the determination of yield potential, the change in maximum photosynthetic rate with genotype, phylogeny and with adaptation to the light intensity under which the leaves were grown was examined in a number of experiments under both natural and artificial light.

The role of cell size increases in determining the increase in leaf and grain size with evolution was studied and the association of mesophyll cell size with photosynthetic rate was examined.

Data from the early part of this work was published during the preparation of this thesis (Evans and Dunstone, 1970), at about the same time as somewhat similar work by Khan and Tsunoda, and has been included as Experiment 1.

MATERIALS AND METHODS

Genetic Materials

Twenty three lines representing nine species of the genera Triticum and Aegilops were used in the series of experiments (Table 2 : 1). Both wild and cultivated species of Triticum were represented at the diploid and tetraploid level, and two species of cultivated wheats at the hexaploid level. Two Aegilops species were each represented by two lines in each experiment. These lines, and a number of others, were originally compared in two exploratory experiments and they were chosen to represent the range of behaviour observed.

With T. boeoticum, for example, six lines were used to cover the range from the small seeded aegilopoides to the larger seeded thaoudar forms. Four of them behaved as spring forms in the preliminary experiments, with no obligate vernalisation requirement (T-6625, T6626, G31 from Iran and Kew (C64 - 146), whereas the other two required vernalisation to flower.

TABLE 2 : 1
Species and Lines Used in the Experiments

Group	Genome	Species	Line Code
Diploid			
Wild	A	<u>Triticum boeoticum</u> Boiss. emend Schiem.	Kew C64.146 T-6625 PBI C64.145 T-6626 TBI G31
	B	<u>Aegilops speltoides</u> Tausch	AS1 6001 G19
	D	<u>Aegilops squarrosa</u> L.	G46 CHBS G90 CHBS
Cultivated	A	<u>T. monococcum</u> L.	W10 W292
Tetraploid			
Wild	AB	<u>T. dicoccoides</u> Korn.	W1043 T-6252
Cultivated	AB	<u>T. dicoccum</u> Schubl.	Khapli W12 W2698
	AB	<u>T. durum</u> Desf.	Kubanka W8 Acme W9
Hexaploid	ABD	<u>T. spelta</u> L.	H2
	ABD	<u>T. aestivum</u> L.	Gabo Late Mexico 120 Cappelle Desprez

Spring forms of the two Aegilops species were unobtainable although some have been recorded (Kihara, Yamashita and Tanaka, 1965).

The other lines requiring vernalisation were T. dicoccoides (W1043) and T. aestivum lines Cappelle Desprez, and Late Mexico 120 which had a marked vernalisation response.

Both lines of T. dicoccoides were of the robust Palestinian type which probably gave rise to T. dicoccum (Harlan and Zohary, 1966).

The three cultivars of T. aestivum were chosen to represent a range of successful soft wheats: Gabo, an Australian spring variety with awnless ears; Late Mexico 120, a semi-dwarf variety with awned ears; and Cappelle Desprez, a high yielding European winter wheat with awnless ears.

Apart from Gabo and Cappelle Desprez, the only lines without well developed awns were the two species of Aegilops.

It would have been preferable to use more than two lines of all species, but the space available under one set of growing conditions and the number of measurements to be made at ear emergence and during the period of grain development precluded more extensive comparisons.

Growing Conditions

All plants for the experiments were derived from seed grown in a phytotron glasshouse held at 21°C for eight hours of daylight and 16°C for the remaining 16 hours. The natural daylength was extended to 16 hours with incandescent lamps of 50f.c. intensity.

For Experiment 1 the seed samples were selected for uniformity of size, weighed and set out for imbibition. More than 50 germinating seeds of each line were planted singly in small peat cups of perlite and vermiculite and held in long days at 21/16°C for a few days. Because many of the lines required vernalisation, all the established seedlings were moved to 8 hour days at 7/4°C day/night temperature under natural light, for a period of ten weeks.

For Experiments 2, 3, 4, 5 and 6 vernalisation was carried out with the imbibed seeds. Seeds were placed on wet filter paper in Petri dishes and immediately set in a refrigerator in the

dark for 7½ weeks at 2°C. The vernalised seedlings (Experiment 1) or germinating seeds, were then planted out into 5 inch pots filled with a mixture of equal parts of perlite and vermiculite and placed in the long day conditions at 21/16°C, either in the phytotron glasshouse (Experiment 1, 2, 3, 4 and 6) or in an LB type artificially lit cabinet (Experiment 5). This unit was lit by 28 140 W VHP warm white fluorescent lamps supplemented by 4 100W incandescent lamps (Morse and Evans, 1962).

The total radiant energy received on the glasshouse roof each day was measured by means of a "Kipp" solarimeter connected to a CSIRO solar-integrator.

All plants were watered in the mornings with Hoagland's No.2 nutrient solution and in the afternoons with water.

Plants were staked as required to prevent lodging and the mainstems were identified by periodic leaf marking.

Measurement of Photosynthesis and Respiration

In every experiment the photosynthesis and respiration rates were measured in the apparatus outlined in Figure 2 : 1. Air was drawn into the system from the roof of the building and short term fluctuations in CO₂ concentration were eliminated by passing the air through a large (44 gallon) stirred mixing drum. The air was then passed through a "Flostat" regulator and a "Gapmeter" 12L flowmeter to the perspex assimilation chamber and returned through a similar flowmeter.

Before the "Flostat" a by-pass line took off a subsample of the incoming air and passed it through a calcium chloride drying column and a 2L "Gapmeter" to a Grubb Parsons SB2 infra-red gas analyser (IRGA). After the return "Gapmeter" a similar arrangement provided a subsample of the return air to the IRGA. Return air not being passed through the IRGA was vented to the atmosphere.

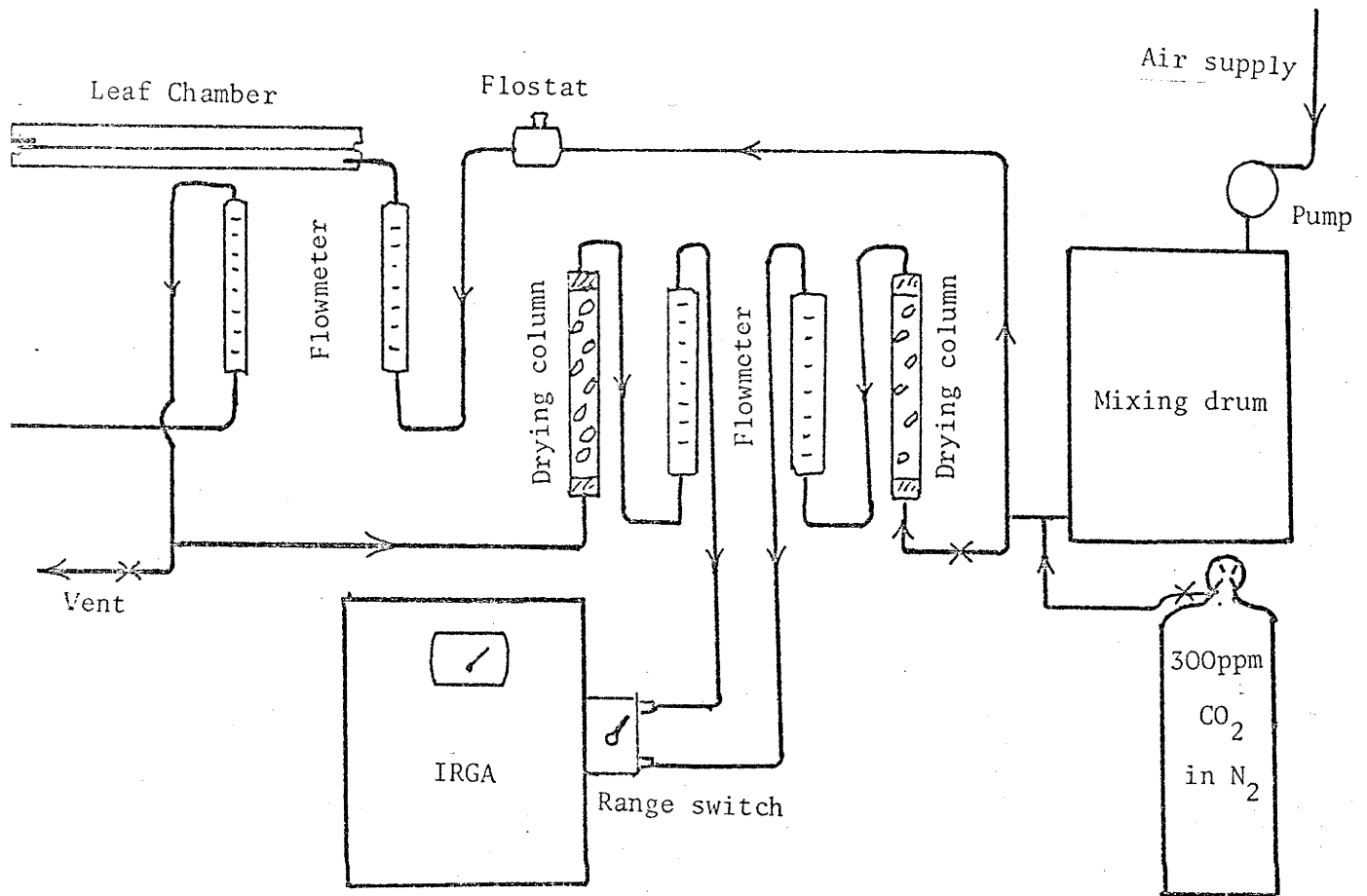


Figure 2 : 1

Apparatus used in the measurement of photosynthesis and respiration.

The range switch on the IRGA was arranged so that the incoming air subsample could be split to pass through both tubes of the IRGA to give a zero. With the switch in this position the air continued to move through the assimilation chamber in a normal manner so that the equilibration of the leaf to the conditions in the assimilation chamber was not disturbed while the zero was being taken.

For Experiment 1 the assimilation chambers used for leaves had a cross section of 2 x 15 cm, that for the ears, 10 x 22 cm.

The flow rate was 4 litres/min. A perspex insert was placed in the leaf chamber for the remainder of the experiments reducing its cross section to 1 x 15 cm and the flow rate was increased to 6 litres/min in order to reduce the boundary layer on the leaves.

The IRGA was calibrated to measure the CO_2 differential against a background of 300 ppm CO_2 in nitrogen using "Wosthoff" gas mixing pumps. A set of three pumps provided gases with CO_2 levels increasing in steps of 10 ± 1 ppm from 0 ppm to 80 ppm, and a calibration curve was drawn.

For Experiment 1, the light up to an intensity of 3200f.c. ($121,600 \text{ ergs cm}^{-2} \text{ sec}^{-1}$ visible) was supplied by the VHO warm white fluorescent light bank supplemented by incandescent lamps of the standard "LB" cabinet (Morse and Evans, 1962). For the high intensities used (7000f.c.; $266,000 \text{ ergs cm}^{-2} \text{ sec}^{-1}$ for Experiments 1 to 4 and 8,600f.c.; $326,800 \text{ ergs cm}^{-2} \text{ sec}^{-1}$ for Experiments 5 and 6) the standard lights were supplemented by a high intensity mercury vapour lamp (Philips HPLR 1000W).

In Experiment 5 a range of low light intensities was obtained by placing layers of white organdy fabric across the top of the assimilation chamber.

Temperature inside the assimilation chamber was regulated by maintaining the cabinet and the incoming air at 21°C . The temperature inside the chamber then stabilised at $25 \pm 1^\circ\text{C}$ as measured underneath the leaf with a thermocouple connected to a Honeywell potentiometric recorder.

In Experiment 1 the net photosynthesis rate was also measured at 3200 f.c. in an atmosphere consisting of 300 ppm CO_2 in nitrogen with 1% O_2 . The enhancement of net photosynthesis when the oxygen content was lowered was taken as an estimate of the rate of light respiration (Hesketh, 1967).

For convenience of measurement the light conditions for all photosynthesis measurements and for Experiment 5 pre-treatments were set up using an "Eel" photometer which was calibrated by the author using a lamp standardised by the National Standards Laboratory and each setting was made in terms of foot candles. So that comparisons can be made with work done under other light sources, the light settings were also measured in terms of light energy in the range 400 to 700 nanometers with a "Kettering" YS1 radiometer and these measurements are expressed in $\text{ergs cm}^{-2} \text{sec}^{-1}$ visible.

Leaf areas for Experiment 1 were obtained by careful use of a "Paton" airflow planimeter which was standardised for a small section of the scale to be used for each set of measurements.

For the remaining experiments the leaf areas were obtained by printing a projection of the leaf onto draughtman's photosensitive paper and weighing the cut out paper image after drying at 80°C in an oven for 24 hours. The weight/area ratio was established under identical conditions.

Specific leaf weight was calculated by dividing the dry weight of the leaf blade (mg) by the area of the leaf blade (cm^2).

^{14}C Distribution

Uptake of $^{14}\text{CO}_2$ and its redistribution after 24 hours was used to compare the patterns of assimilate distribution during the period of most rapid grain growth in Experiment 1. This period varied to some extent between lines, but a standard time of exposure was used, namely 14 days after anthesis. In separate lots of eight plants either the flag or the penultimate leaf was exposed to $^{14}\text{CO}_2$, the plants being harvested 24 hours after the initial exposure.

$^{14}\text{CO}_2$ was generated by addition of 50% lactic acid to barium carbonate (1 mCi/m mole) used at the rate of 2 mg per plant. The course of $^{14}\text{CO}_2$ uptake under light at 3200 f.c. was monitored by a gas flow scintillation cell (IDL 663).

At harvest the plant parts were separated and a Geiger-Muller tube was used to make a preliminary examination of the distribution of radioactivity. The parts were then dried, weighed and ground in a Wiley Mill to pass through a 40 mesh seive. The samples were thoroughly mixed and constant volume sub samples of about 30mg were taken. These were packed into solid planchets of a standard geometry compressed with a tool designed to pack the powder a standard distance of 0.5 mm below the lip. The thickness of the sample was such that "infinite thickness" laws applied. The samples were then counted in a "Tracerlab" automatic counter. The relative total specific activity of the plant parts were calculated by multiplying the relative activity of the sub sample of 30 mg by the dry weight (O'Brien and Wardlaw, 1961).

Weights and Volumes of Grains

In Experiment 6 the weights and volumes of the grains were obtained so that any change in density with increase in grain size could be evaluated. At maturity the basal grain from each of the two centre spikelets on each side of the main stem spike was harvested and dried at room temperature (22°C) for two weeks. The grains were then weighed and the volume calculated by a water displacement method.

A Pasteur pipette was marked about 5 cm above the neck. The pipette was then filled with water to the mark and the water run out into a weighed tube. The four grains were then placed in the pipette and the water again drawn up into it from the tube as far as the mark. The volume of water remaining in the tube was then evaluated

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by weighing.

Nitrogen Assay

A selection of eight large grains were used to determine the nitrogen content by a micro Kjeldahl method.

Sectioning and Staining

Material to be sectioned was fixed and stored in formalin-acetic acid-alcohol made up of 90 parts of 70% ethanol, 5 parts formalin 5 parts acetic acid.

The material was washed for eight hours in tapwater then soaked in 10% v/v aqueous glycerol overnight. In the morning the material was embedded in a matrix consisting of 15% gelatine (w/v) with 2% glycerol (v/v) (Knox, 1970).

Blocks were then cut from the gelatine and quickly frozen on the rapid freeze stage of a cryostat using a liquid "Freon 12" aerosol to accelerate the process. Sections were then cut on the cryostat microtome at -15°C .

For Experiment 5 cross-sections of the leaves were cut $10\text{ }\mu$ thick. These were stained first with 1 : 4 Harris Haemotoxylin : safranin, then with Analine blue (1% solution in 95% ethanol). The sections were mounted in "Eukitt" as permanent slides.

For Experiment 6 leaf sections were cut from the centre portion of the leaf $6\text{ }\mu$ thick in three planes, cross sectional longitudinal and tangential. Grains were sectioned at $10\text{ }\mu$ thick in the median cross-sectional and longitudinal planes.

All leaf sections and one set of grain sections were stained with Sharman's tannic acid, orange G stain. The second set

of grain sections was stained with Ponceau 2R xyloidine red stain which stains areas of protein accumulation (Flint and Moss, 1970). The sections were permanently mounted in "Eukitt" medium.

Mesophyll Cell Separation

In Experiment 6 mesophyll cells were separated out into a suspension so that examination and measurement of single whole cells could be made. A central portion of the fixed leaf material of each line was washed in tapwater for 1 hour, then stained in Feulgan's for $\frac{1}{2}$ hour. The portion was then rinsed in tapwater and immersed in a .1% solution of cellulase in pH 5.0 citrate buffer at 40°C for 4 hours. It was then rinsed in tapwater and teased out with needles in 50% aqueous glycerol under a dissecting microscope. A Pasteur pipette was used to further separate the cells by rapid syringing of the suspension. Temporary slides were made up by placing a drop of the suspension on a microscope slide and covering it with a cover slip.

Microscopic Measurements, Counts and Tracings

All cell counts and cell tracings were taken directly from the screen of a "Reichhardt" lanameter (screen microscope). The scales or fields used were calibrated with a "Zeiss" stage micrometer.

Measurements of leaf thickness were made at 4 places across the leaf for each of 4 leaves for each line. The measurements were made over small vascular bundles, avoiding the mid-rib area and the edges of the leaf.

The cross sectional area of the endosperm cells of the grains was calculated by counting the number of cells in each of 4 fields of .38 mm diameter selected across one lobe of the grain. Two sets of 3 fields were counted for each of 9 grains for each line.

In order to obtain the projected area of the separated mesophyll cells, the outlines of 15 cells for each of 9 leaves of each line were traced from the screen of the lanameter onto clear plastic sheets. The tracings were then printed onto draughtman's photosensitive paper and the images were carefully cut out and their areas measured by a planimeter. The projected area was then calculated using the calibration figure.

Stomatal Frequencies

Impressions were made of the upper and lower surfaces of the leaves with a fast setting, low viscosity silicone rubber compound (Dow Corning RTV583 with No.4 catalyst) according to the method of Sampson (1961).

Replicas of the rubber impressions were then made by painting the rubber with clear nail polish and allowing it to dry. The lacquer was then peeled off using a piece of clear cellulose tape which was then mounted on a microscope slide.

In all cases care was taken that the slide obtained was an impression of the leaf half way along its length where stomatal frequency is at about the mean value for the whole leaf (Pazourek, 1969).

Counts were made of the number of stomata falling within a measured field of the Reichhardt lanameter. Six fields were counted across the upper surface and six across the lower surface of each leaf. The mean number of stomata per cm^2 was then calculated.

EXPERIMENT 1

Growth, photosynthesis and translocation

Introduction

This experiment was planned to examine, on a fairly wide basis, the capacity or potential of processes that may limit yield. An examination was carried out of growth both at the vegetative stage and after anthesis. Concurrently a number of systems was studied; photosynthesis and respiration of leaves and ears and the distribution of assimilates from various leaves. The data from this experiment were published during the preparation of this thesis (Evans and Dunstone, 1970)*, but it is included here because this experiment is the basis for the following experiments and because some of the data are placed alongside data from later experiments in the concluding chapter of the thesis.

Materials and Methods

The lines used in this experiment are as listed in Table 2 : 1, except for the omission of G19 and G31.

The plants were vernalised and grown under natural light conditions as previously described.

At the time of the experiment the natural (summer) days were about 14 hours in duration and constantly sunny, and the total radiation received during ear development averaged $614 \text{ g. cal cm}^{-2} \text{ day}^{-1}$.

The plants were arranged in blocks. These were rearranged weekly to minimise variation due to shading etc.

Rates of tillering and of leaf area increase were measured over a period of about 3 weeks following vernalisation. They were also measured in separate lots of 16 plants grown without vernalisation at $21/16^{\circ}\text{C}$. With the vernalised plants, examination of apices by dissection, 7 days after returning to long days at $21/16^{\circ}\text{C}$, indicated that all lines had either initiated inflorescences,

* See acknowledgements

or were elongating and approaching initiation. Anthesis in all lines occurred within a period of about 2 weeks duration.

For each line, groups of 7 or 8 plants were harvested at ear emergence, at 5, 15 and 21 days, after anthesis, and at maturity of the main stem ear.

Prior to all harvests except that at maturity, the rates of photosynthesis and dark respiration by the ear and the flag leaf blade on the main stem were measured.

The pattern of distribution of the ^{14}C -labelled assimilates was examined in plants exposed to $^{14}\text{CO}_2$ 14 days after anthesis and harvested the following day i.e. 24 hours after exposure to $^{14}\text{CO}_2$.

At harvest all plants were divided into grains, ear structure, flag and other leaves (including sheaths); top, second and other internodes of the main stem and tillers. All parts were dried at 80°C in a forced draught oven for 48 hours and weighed.

At the end of the experiment two plants representative of each line were dried and pressed to be held as type specimens.

Measurement of Photosynthesis and Respiration

At all harvests measurements of photosynthesis at 3200f.c. ($121,600 \text{ ergs cm}^{-2} \text{ sec}^{-1}$) and dark respiration by the ear and the flag leaf blade were made. Plants were equilibrated under these conditions for at least one hour prior to measurements, which were made during the morning on two lots of four leaves or ears of each line. At ear emergence, rates of flag leaf photosynthesis were measured at 1,000, 2,000, 2,500, 3,200 and 7,000f.c. (38,000, 76,000, 95,000, 121,600, 266,000 $\text{ergs cm}^{-2} \text{ sec}^{-1}$).

Five days after anthesis the rate of flag leaf photosynthesis was measured at 3,200f.c., and an estimate of the rate of photorespiration made.

RESULTS

(a) Growth Measurements

(i) Leaf Areas

During early growth the total leaf area of unvernalsed seedlings of the various lines was approximately proportional to the weight of the grains which were sown (see Figure 3 : 1). Seedling establishment was slowest in the diploids with the smallest grains, especially A. speltoides, and was most rapid in the tetraploids and the hexaploids. During early growth, there were only slight differences between lines in the rates of leaf appearance, and the differences in total leaf area in Figure 3 : 1 were due mainly to differences between lines in the size of individual leaves. Relative growth rates for leaf area between 11 and 27 days from sowing did not differ markedly between species (Table 3 : 1, column 5), and diagrams presented by Kranz (1966) support this conclusion.

Successive leaves were progressively larger in all lines except that the flag leaf blade was smaller than that of the penultimate leaf in T. boeoticum, T. monococcum, and T. spelta, being particularly reduced in T. boeoticum in which the area of the flag leaf was less than half that of the leaf below it.

TABLE 3:1

SOME CHARACTERISTICS OF WHEAT AND RELATED SPECIES
Each value is the average for all lines of the species. Further details in text

Species	(1) Spikelet Number per Ear	(2) Grain Number per Ear	(3) Weight per Grain (mg)	(4) Area of Largest Leaf (cm ²)	(5) Leaf Area Growth Rate (cm ² /cm ² /day)	(6) Maximum Grain Growth (mg/ear/day)	(7) Grain Weight as % of Main Stem Weight	(8) No. of Tillers with Ears at Final Harvest	(9) Stem Weight Loss (mg)	(10) (11) Photosynthetic Rate at 3200 f.e. Flag Leaf* Rate	(12) Rate
<u>boeoticum</u>	18.1	30.4	13.6	8.8	0.173	25.1	33.8	34.3	59	45.7 (18.8)	2.7
<u>monococcum</u>	22.1	21.4	32.0	19.1	0.184	31.8	39.6	46.4	69	31.9 (13.0)	1.8
<u>speltoides</u>	9.1	11.6	4.6	5.0	0.179	3.7	9.5	71.4	48	33.8 (9.6)	1.1
<u>quarrosa</u>	10.2	19.5	10.0	8.2	0.190	10.8	31.5	43.3	47	36.4 (13.1)	1.7
<u>dicoccoides</u>	14.3	26.8	32.9	34.5		40.2	36.3	19.6	80	34.6 (14.4)	6.4
<u>dicoccum</u>	20.0	37.1	35.8	29.2	0.161	44.3	39.0	18.5	210	29.5 (11.0)	4.1
<u>arabum</u>	19.2	47.2	34.7	31.2	0.196	48.2	38.1	10.8	31	28.8 (12.9)	6.3
<u>spelta</u>	22.4	35.6	56.1	40.8	0.179	48.5	36.2	16.1	148	27.3 (10.8)	4.5
<u>estivum</u>	17.0	36.0	49.0	28.6	0.176	57.1	49.3	14.1	198	31.4 (14.7)	3.3

* Photosynthetic rate for flag leaf in air expressed as mg CO₂/dm²/hr. Values in parenthesis show increase in photosynthetic rate for flag leaf when oxygen is excluded.

† Gross photosynthetic rate expressed as mg CO₂/ear/hr.

(ii) Tillering

Lines differed to some extent in their early tillering rates, but after inflorescence initiation only a few additional tillers were formed in the tetraploid and hexaploid wheats whereas the diploids, especially A. speltoides, continued tillering at a high rate until maturity, resulting in very great differences in final number of tillers bearing ears (Table 3 : 1, column 8). The high tiller number in many of the diploids compensated for their smaller leaves and more slender stems. Dry weights at final harvest depended on the time to maturity of the main ear and on whether tillering continued, being highest in Cappelle Desprez, T. monococcum W292, and A. speltoides AS1. At maturity plants of Cappelle Desprez had 15.1 tillers, those of AS1 84.1.

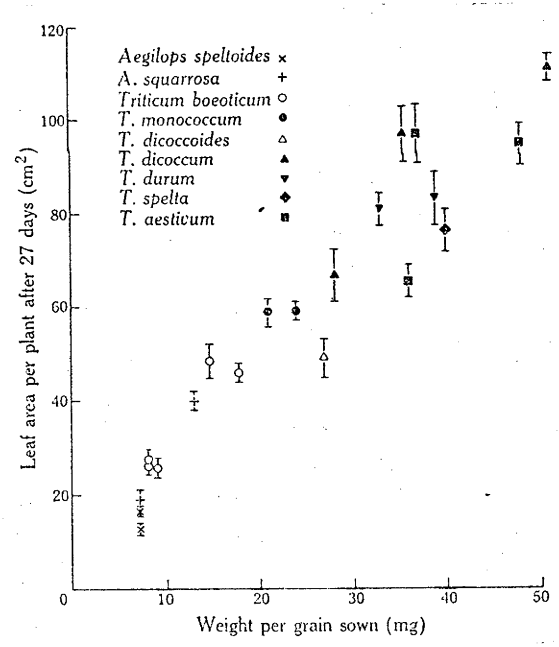


Figure 3 : 1

Relation between the average leaf area for seedlings of each line 27 days after germination and the average weight of the grains sown. The vertical line represents 2 x S.E.

(iii) Stem Weight Losses

The weight of the main stems varied greatly between lines depending mainly on their height. They were heaviest in T. durum and T. spelta, lightest in A. squarrosa. There was also considerable variation in the time at which stem weight reached its maximum, this being 5 days after anthesis in some lines (e.g. T. dicoccum, W12), 15 days after in many (e.g. Cappelle Desprez), 21 days after in several (e.g. T. durum, W9), and at maturity in T. durum (W8).

The average losses in stem weight between the maxima and the final harvests are given in Table 3 : 1, column 9. They were greatest in T. dicoccum and the hexaploid wheats, least in T. durum and the wild diploids.

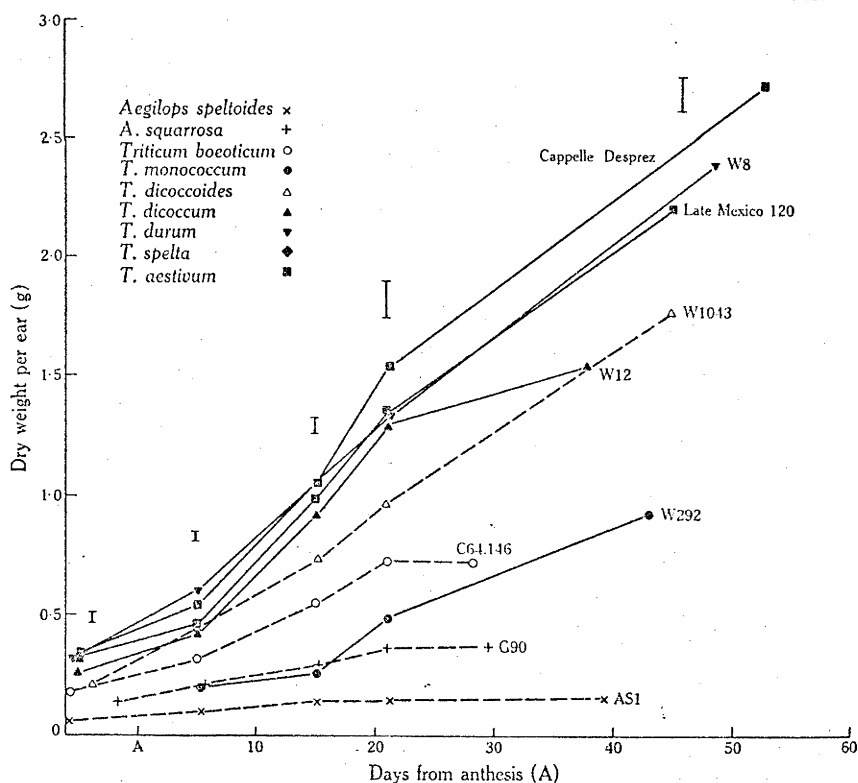


Figure 3 : 2

Changes in dry weight of main stem ears with time from anthesis, in some representative lines. The vertical lines represent 2 x mean S.E. for each harvest.

(iv) Ear Characteristics

-Changes in ear weight on the main stem with time after anthesis in some representative lines are shown in Figure 3 : 2. Grains were separated from the ears of all lines in the three last harvests, and grain weights increased in much the same way as ear weights. The weight of the ear structures (i.e. ear minus grains) remained fairly constant or increased to a small extent in some lines at the final harvest; they were smallest in A. speltoides, heaviest in T. dicoccoides and T. spelta. Figure 3 : 2 illustrates the very great differences between lines in the course of ear growth. Final grain weight per ear was least in the wild diploids, and tended to increase with increase in ploidy, and with selection for cultivation. However, there were exceptions in that final grain weight in the wild T. dicoccoides (W1043) exceeded that in the cultivated T. dicoccum (W12), while the tetraploid T. durum (W8) exceeded the hexaploid Gabo and Late Mexico 120. The highest rate of grain growth measured increased with both ploidy and cultivation, as may be seen from Table 3 : 1, column 6. Grain growth began later but continued for longer in both lines of the cultivated T. monococcum compared with that in all lines of the wild T. boeoticum. At the tetraploid level, however, grain growth continued for longer in the wild T. dicoccoides (W1043) than in the earlier-maturing T. dicoccum (W12).

Spikelet number per ear in Aegilops spp. was much lower than in Triticum spp. in which, under our conditions, there was no evidence of any increase with evolutionary level (Table 3 : 1, column 1). However, vernalisation can reduce spikelet number in hexaploid wheat to a considerable extent (Rawson, 1970) and may therefore have limited expression of the differences between the lines used in our experiment.

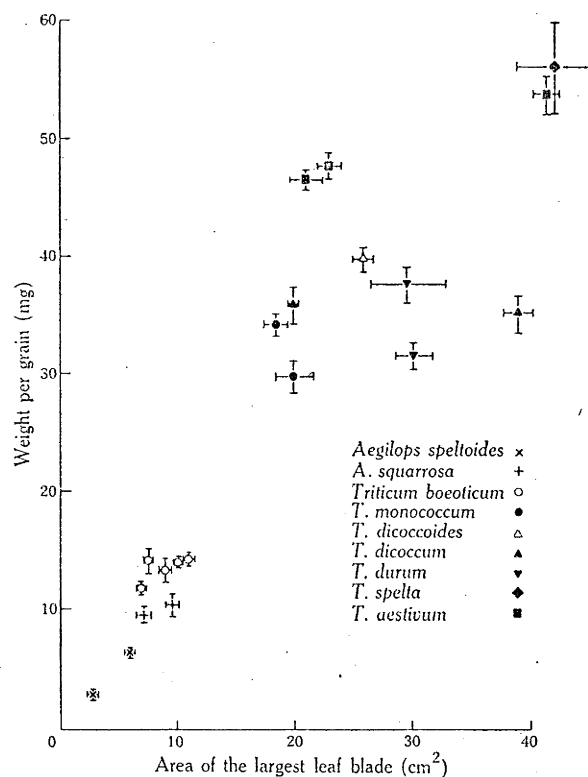


Figure 3 : 3

Relation between mean weight per grain in main stem ears and the area of the largest leaf blade. The vertical line represents 2 x S.E. The horizontal line represents the range.

Grain number per ear (Table 3 : 1, column 2) was lowest in *A. speltoides*, which bore only about one grain per spikelet, and in *A. squarrosa* which bore about two. In the five lines of *T. boeoticum* grain number per spikelet ranged from 1.46 to 1.87, whereas in both lines of *T. monococcum* it was slightly less than one.

Individual grain weight varied greatly between species (Table 3 : 1, column 3) being lowest in *A. speltoides* and highest in the hexaploids. The values we obtained were similar to those given for a few of the species by Percival (1921) and Bell, Lupton and Riley (1955). There was a 20-fold range between individual lines in

mean weight per grain, which bore a close relation to the area of the largest leaf blade (Figure 3 : 3). Differences in weight per grain accounted for most of the differences between lines in grain yield per ear, which was also closely related to the area of the largest leaf (Figure 3 : 4). The smaller number of grains per ear in T. monococcum compared with T. boeoticum was more than compensated by the 2.5-fold increase in mean grain size. Grain size in the tetraploids was greater than that in the diploids, but was greater in one genotype of the wild T. dicoccoides, W1043, than in the cultivated forms, particularly those of T. durum, in which there were many more grains per ear. Grain size was still greater in the hexaploids, being greatest in T. spelta and in Cappelle Desprez. The proportion of main shoot weight (including leaves) represented by the grain is given in Table 3 : 1, column 7. It was particularly low in both lines of A. speltoides, and highest (57.1%) in the semi-dwarf hexaploid Late Mexico 120.

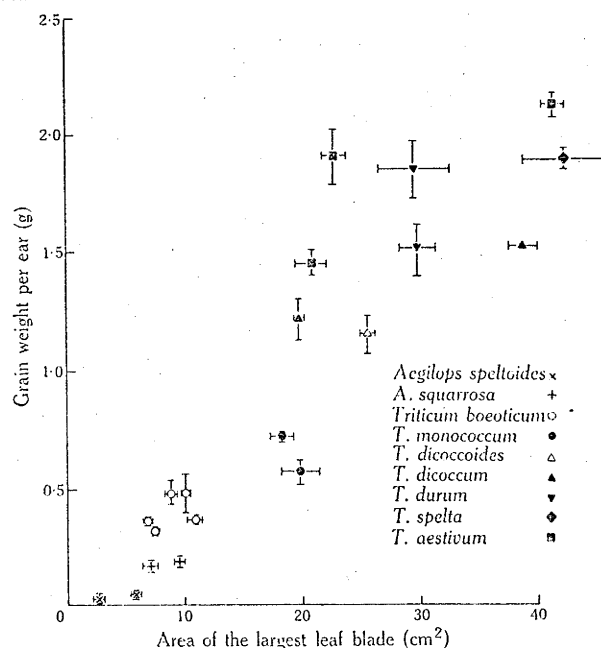


Figure 3 : 4

Relation between the average grain weight in the main stem ear of each line and the area of the largest leaf blade. The vertical line represents 2 x S.E. The horizontal line represents the range.

(b) Photosynthesis

The rate of photosynthesis per unit flag leaf area, at high light intensity, has apparently fallen in the course of evolution of wheat (Figure 3 : 5). The highest rates were found in T. boeoticum, and A. squarrosa (G46), the lowest, less than half those of T. boeoticum, in Cappelle Desprez. The rates were inversely related to the area of the flag leaf blade.

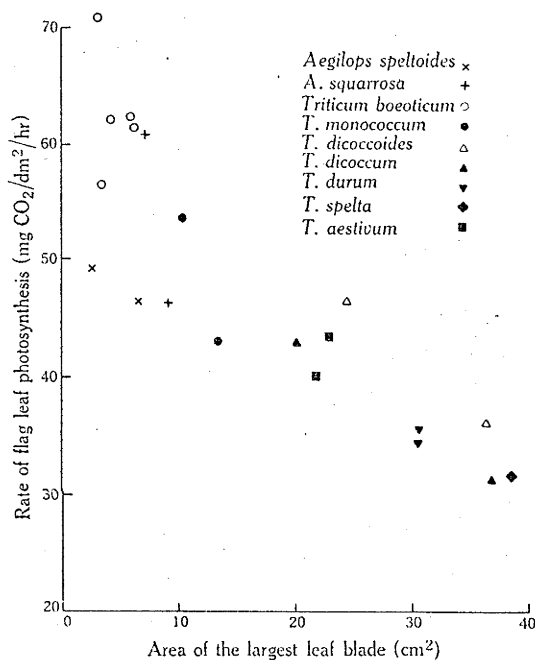


Figure 3 : 5

Relation between the rate of photosynthesis by flag leaf blades, at the time of ear emergence, in light of 7000 f.c. intensity at atmospheric CO₂ concentration, and the area of the flag leaf blades.
 $r = .91$ $P < .001$.

At lower light intensities the differences between species were less pronounced (Figure 3 : 6), and gross photosynthesis (i.e. net photosynthesis plus dark respiration) at an intensity of 1000f.c. ranged from only 16.4 mg CO₂ dm⁻² hr⁻¹ for T. aestivum to 19.1 for T. boeoticum. At an intensity of 3200 f.c. net photosynthesis by flag leaves of T. aestivum, T. spelta, and T. durum

was approaching light saturation, whereas in T. boeoticum and A. squarrosa it increased by a further 50% with increase in light intensity to 7000 f.c. The average rates at 3200 f.c. are given in Table 3 : 1, column 10, together with the increase in photosynthetic rate at 3200 f.c. when oxygen is excluded (column 11). This estimate of the rate of photorespiration indicates that the high rates of net photosynthesis in the wild progenitors are not due to low rates of photorespiration. In fact these were also highest in T. boeoticum. The differences in flag leaf photosynthetic rates between species were also apparent between rates measured on the penultimate leaf.

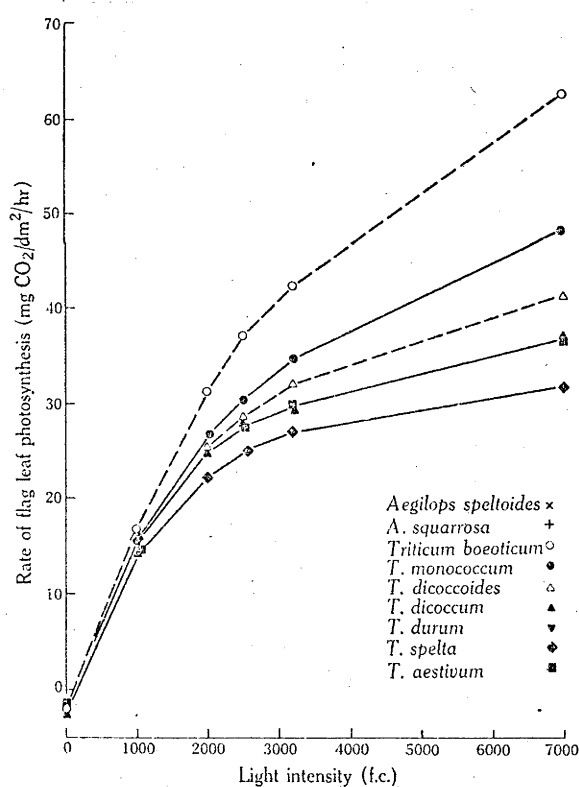


Figure 3 : 6

Relation between photosynthetic rate of flag leaf blades and light intensity in several species. Each curve is the average for all lines.

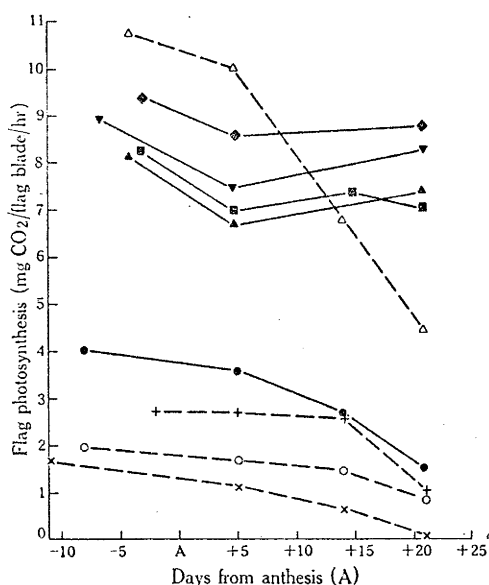


Figure 3 : 7

Changes with time from ear emergence in photosynthesis per flag leaf blade, at 21°C in light of 3200 f.c. intensity. Symbols as in Figure 3 : 6.

The larger area of the flag leaves of the more advanced wheats more than compensated for their lower rates of photosynthesis, as may be seen from Figure 3 : 7. Photosynthesis per flag leaf blade was initially greatest in the wild T. dicoccoides, and was about four times as great in the cultivated tetraploids and hexaploids as in T. boeoticum, which had the highest rate per unit leaf area. In addition to this major difference between the diploids and the other species, there was a further difference in the changes with time from ear emergence. In all the wild species, and in T. monococcum, flag leaf photosynthesis fell progressively throughout the period of grain development, whereas in all the cultivated tetraploids and hexaploids it remained high until at least 21 days after anthesis, in fact was higher then than 5 days after anthesis.

This rise in the rate of flag leaf photosynthesis during rapid grain growth has been examined in more detail elsewhere (Evans and Rawson, 1970; Rawson and Evans, 1971) and is probably associated with increased demands for assimilates by the developing ears.

PERCENTAGE DISTRIBUTION OF ^{14}C -LABELLED ASSIMILATES 1 DAY AFTER EXPOSURE TO $^{14}\text{CO}_2$
14 DAYS AFTER ANTHESIS

Species	Percentage ^{14}C in:						Tillers
	Fed Leaf	Ear	Top Stem Inter-node	Second Inter-node	Basal Inter-node	Roots	
Flag leaf exposed to $^{14}\text{CO}_2$							
<i>T. boeoticum</i>	21.4	40.3	5.1	9.8	4.2	2.1	16.9
<i>T. monococcum</i>	25.0	23.0	9.5	14.9	8.2	2.8	16.2
<i>A. speltoides</i>	22.0	3.7	4.1	14.6	9.1	14.5	31.9
<i>A. squarrosa</i>	30.1	39.9	6.4	7.2	2.8	2.3	11.6
<i>T. dicoccoides</i>	31.0	28.6	10.5	9.6	2.9	1.8	15.3
<i>T. dicoccum</i>	33.5	52.8	1.4	1.5	0.5	0.9	9.1
<i>T. durum</i>	22.5	44.9	12.2	6.0	1.7	0.9	11.3
<i>T. aestivum</i>	24.0	52.4	5.7	2.5	0.4	1.0	11.4
Penultimate leaf exposed to $^{14}\text{CO}_2$							
<i>T. boeoticum</i>	26.3	23.7	1.3	3.6	11.4	5.5	27.7
<i>T. monococcum</i>	33.0	3.7	1.5	5.7	28.3	6.7	20.6
<i>A. speltoides</i>	32.7	1.0	1.4	2.1	15.5	6.4	39.9
<i>A. squarrosa</i>	37.3	6.8	0.6	3.6	27.2	9.1	14.0
<i>T. dicoccoides</i>	36.1	5.7	0.6	2.6	11.5	4.1	38.6
<i>T. dicoccum</i>	41.3	19.4	0.5	2.2	9.4	3.2	22.5
<i>T. durum</i>	29.2	16.1	3.5	7.8	21.9	3.9	15.9
<i>T. aestivum</i>	25.5	23.9	2.7	11.1	17.0	3.1	14.2

TABLE 3 : 2

Rates of gross ear photosynthesis (Table 3 : 1, column 12) differed considerably between lines, being lowest in *A. speltoides* and highest in the heavily awned ears of *T. dicoccoides* and *T. durum*. The differences between species appeared to be determined mainly by ear size and awn length.

Spikelet number was similar in Gabo and Late Mexico 120 whereas gross ear photosynthesis was 2.1 mg CO₂ per ear per hour in the awnless Gabo and 5.2 mg in the awned Mexico.

(c) Distribution of ¹⁴C-labelled Assimilates

Of the ¹⁴C assimilated, the proportion exported within 24 hr was rather higher for flag leaves than for the penultimate leaves (see Table 3 : 2), and was differently distributed, much more flag leaf assimilate being found in the ear and less in the stems, roots, and tillers.

The species differed considerably in their partitioning of assimilates during ear development. Evolutionary changes are discernible but they are complicated by other factors, such as the relative size of the flag leaf, the magnitude of ear photosynthesis, and the time of exposure to ¹⁴CO₂ in relation to the course of grain growth. For example, it can be seen from Figure 3 : 2 that grain growth in T. monococcum (W292), as also in W10, was extremely slow at the time of exposure to ¹⁴CO₂, 14 days after anthesis, whereas that in T. boeoticum (C64.146), and also in the other lines, was much faster. This, and the relatively smaller flag leaves of T. boeoticum, probably account for the much higher proportion of labelled assimilates translocated to the ear in T. boeoticum compared with T. monococcum. Similarly, the rather high proportion of assimilates from the flag leaf, compared with that from the penultimate leaf, translocated to the ears of A. squarrosa may reflect the small size and early senescence of the flag leaves in that species.

With these effects in mind, the data of Table 3 : 2 suggest that evolution in wheat has been accompanied by an increase in the proportion of assimilates translocated to the grain. The ears of A. speltoides were largely self-supporting for assimilates;

those of T. monococcum and T. dicoccoides imported about one-quarter of the flag leaf assimilate, but little from the leaf below; while those of T. dicoccum and T. aestivum, with faster grain growth and less ear photosynthesis to support it, imported more than half of the flag leaf assimilates, and almost a quarter of those of the penultimate leaf, within 24 hr.

The proportion of labelled assimilate translocated to the roots is low at this late stage of development of the plants, but was higher in the diploids (particularly the two species of Aegilops) than in the modern wheats. The proportion in the tillers was also lower in the modern wheats.

The partitioning of assimilates within the stem also appears to have changed progressively in the course of evolution. In the diploids most of the activity from the flag leaf was found in the penultimate and lower internodes, while that from the penultimate leaf was predominantly in the lowest internodes. In the tetraploids and hexaploids, on the other hand, very little activity from the flag leaf was found at the base of the stem, most being in the top internode, and a much higher proportion of that from the penultimate leaf was also found in the upper internodes. This change reflects the increased tendency towards upwards movement of assimilates in the more advanced wheats, which is coupled with a progressive reduction in the proportion distributed to the roots and tillers. It also suggests that the transport of assimilates from the flag leaf to the ear may follow a more direct route in the modern wheats, joining the upward flow at the uppermost node, whereas in the diploids it may, as Percival (1921) suggests, first move down to the penultimate node.

Preliminary Discussion

There have been marked increases in both grain size and leaf size with evolution in wheat. The larger the grain sown the faster was seedling establishment (Figure 3 : 1) and the larger the individual leaves. In turn larger leaves were associated with larger grains in the ear. Whether these parallel changes reflect a common basis, such as increasing cell size, is not yet known. Schwanitz (1966) observed that cultivated plants generally had larger organs than their wild progenitors and that these "gigantic" organs were associated with large cells. The role played by increase in cell size in the parallel increase in size of grains and of leaves with evolution will be investigated further (Experiment 6).

The rate of photosynthesis 'per unit' flag leaf area was similar for all species when measured at low light intensities, but at high intensities much higher rates were measured in the more primitive species (Figure 3 : 5 and 3 : 6). At a light intensity of 7000 f.c. the photosynthetic rate of T. boeoticum (6625) was 2.6 times as high as that in Cappelle Desprez.

Khan and Tsunoda (1970a) also found that the wild diploid species had the highest rates of photosynthesis and that wild species had higher rates than cultivated species at the diploid and tetraploid levels. Although the ranking of the rates of photosynthesis agreed with the ranking in the present work, Khan and Tsunoda (1970a) reported lower absolute values. This could be due to different conditions within the leaf chamber during measurement or different conditions during the period of growth and development.

The rates of photosynthesis measured for the Aegilops species in the present work were similar to those obtained by

Naaber (1964) under comparable conditions apart from a higher CO₂ concentration.

However, Belikov et al (1961) found no difference in photosynthesis rates between T. monococcum, T. dicoccum and T. aestivum even at high light intensities. Their measurements were made only on the second leaf of young plants, and it may be that early leaves do not display the same rates of photosynthesis as later leaves. This point is to be investigated further (Experiment 2).

EXPERIMENT 2

Photosynthesis, natural light - winter conditions

Introduction

In Experiment 1 it was observed that the rate of photosynthesis has fallen with evolution, a conclusion also reached by Khan and Tsunoda (1970a).

In contrast, Belikov et al (1961) found no differences between the photosynthetic rates of cultivated species at three levels of ploidy when they measured the rates of the second leaf developed on young plants under both high and low light intensities.

Experiment 2 was planned to measure the photosynthetic rates of successive leaves of the developing plants and to measure a number of morphological and anatomical features which may be significant in explaining the trends observed.

Materials and Methods

The lines used in this experiment were the same as for Experiment 1 with two lines omitted, 6001 (Ae. speltoides) and T-6252 (T. dicoccoides).

The imbibed seeds were vernalised and the plants grown under glasshouse conditions as previously described.

Photosynthesis measurements began with the fifth leaf developed and continued with successive leaves to the flag leaf.

Since different lines developed different numbers of leaves, the data were arranged according to the leaf position counting from the flag leaf. In the results the data for lines is compared for the flag leaf (F), one below the flag leaf (F-1) and sequentially to (F-3). All measurements were made at 7000 f.c. A leaf was considered ready for measurement when it had fully expanded and the following leaf (or the ear) had about half emerged.

Measurements began on 4th May. During the development of the leaves

the weather was frequently overcast and the average total daily radiation received during the period of measurement was $230 \text{ cal cm}^{-2} \text{ day}^{-1}$.

The photosynthesis measurements were made on two groups of four plants for each leaf of each line. One group of four plants was then used to make a set of silicone rubber impressions of the epidermis. After the rubber was peeled off, the leaf blades were cut off and dried so that the specific leaf weight could be calculated.

The second group of four leaves was fixed in FAA for subsequent sectioning.

RESULTS

The mean photosynthesis rate for each leaf of each species is shown in Figure 4 : 1. For most species the flag leaf rate was as high as, or higher than, the rate of any earlier leaf measured. Both Aegilops spp. however had higher rates for (F-2) than for the flag leaf. T. boeoticum and T. spelta did not have the sharp increase from (F-1) to the flag leaf which was common to all other species.

The pattern of photosynthesis data for T. spelta was different from the pattern of all other species. The rate for (F-1) was lower than for (F-2), while the flag leaf blade had a rate lower than any other leaf measured.

The maximum rate obtained was for the flag leaf of the wild tetraploid T. dicoccoides which reached a rate of $45.5 \text{ mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$. The lowest rate recorded was also for a flag leaf of the cultivated hexaploid T. spelta which had a rate of $25.7 \text{ mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$.

The difference between species in photosynthesis rates of the various leaves ranged from $12 \text{ mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$ for leaf (F-3)

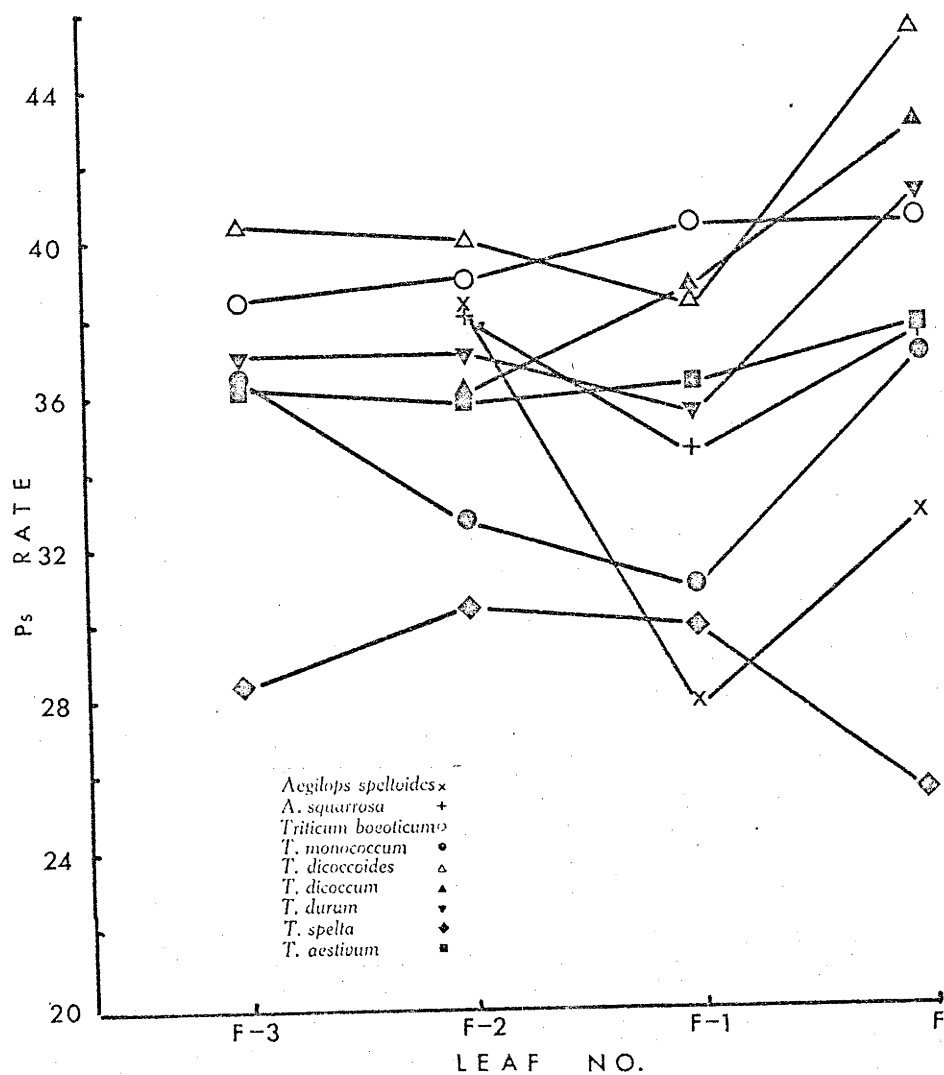


Figure 4 : 1

Graph showing the photosynthetic rate (Ps) at 7000 f.c. and atmospheric (CO₂) in mg CO₂ dm⁻² hr⁻¹ for successive leaves, from the third leaf below the flag leaf (F-3) to the flag leaf (F). Each point is the mean for the species, calculated from the measurement of two groups of 4 leaves of each line of that species.

to more than $19 \text{ mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$ for the flag leaf. Most of the spread in the ranges of rates of (F-3) was due to the low value of one species, T. spelta. The range of rates for species other than T. spelta increased from less than $5 \text{ mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$ for (F-3) to more than $12 \text{ mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$ for the flag.

Leaf Area

In all lines leaf area rose from (F-3) to (F-1). (Table 4 : 1, LA). In both diploid Triticum spp. and in the wild tetraploid T. dicoccoides, the leaf area fell from (F-1) to F, while for all other species the leaf area rose from (F-1) to F.

Considering all leaves, no relationship was observed between leaf area and photosynthesis rate. When the flag leaves were graphed separately, the strong negative correlation observed in Experiment 1 no longer held for this experiment. The breakdown of the relationship was associated with a marked reduction in photosynthesis rates of all diploid species, while the tetraploid and hexaploid species maintained the relationship observed in the previous experiment (Figure 4 : 2).

Table 4 : 1

	Leaf F-3				Leaf F-2				Leaf F-1				Leaf F			
	Ps	LA	SLW	SF	Ps	LA	SLW	SF	Ps	LA	SLW	SF	Ps	LA	SLW	SF
<u>T. boeoticum</u>	38.4	8.7	2.87	6097	39.0	10.6	2.95	5880	40.5	14.5	3.00	6194	40.4	7.4	3.26	7353
<u>T. monoccum</u>	36.4	11.6	2.65	4791	32.7	20.3	2.22	4955	30.5	23.0	2.36	5925	37.1	17.6	3.14	7460
<u>Ae. speltooides</u>					38.3	4.9	2.37	5666	27.9	6.6	2.54	5807	32.8	8.5	2.75	7201
<u>Ae. squarrosa</u>					38.0	6.5	2.52	5516	34.5	9.3	2.37	5896	37.8	9.7	2.81	6652
<u>T. dicoccoides</u>	40.3	9.7	2.61	3611	40.0	20.6	2.87	4153	38.2	32.2	3.18	5401	45.5	22.6	3.86	6424
<u>T. dicoccum</u>					36.5	15.7	2.32	3414	38.9	24.6	2.52	4194	43.0	30.3	3.27	5277
<u>T. durum</u>	37.0	13.7	2.42	3388	37.4	19.8	2.37	3458	35.6	29.5	2.54	4211	41.2	37.6	3.40	5637
<u>T. spelta</u>	28.7	29.9	2.41	3177	30.6	32.3	2.19	3181	30.0	55.0	3.18	4218	25.7	55.2	3.37	4789
<u>T. aestivum</u>	36.2	16.1	2.54	3147	35.9	16.9	3.19	3723	36.2	29.2	3.18	4316	37.8	44.5	3.83	4768

Ps Photosynthesis rate at 7000 f.c. mg CO₂ dm⁻² hr⁻¹

LA Leaf area cm²

SLW Specific leaf weight mg cm⁻²

SF Stomatal frequency. Number stomata cm⁻²

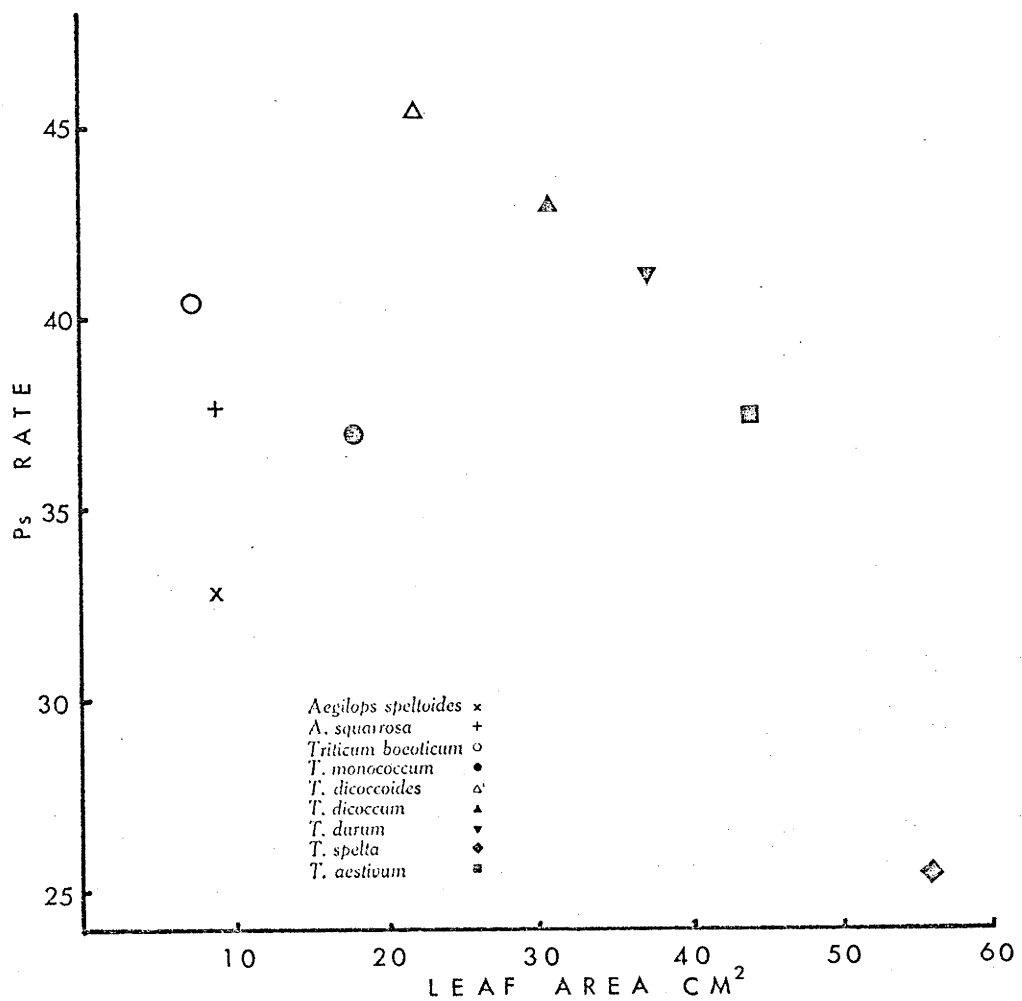


Figure 4 : 2

Relationship between the rate of photosynthesis of the flag leaf blades at 7000 f.c. and atmospheric (CO_2), ($\text{mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$) and the area of the flag leaf blade (cm^2). Each point is the mean for the species, calculated from the measurement of two groups of 4 leaves of each line of that species.

Specific Leaf Weight

Specific leaf weight (SLW) ranged from 2.16 mg cm^{-2} for the leaf F-2 of *T. spelta* up to 3.86 mg cm^{-2} for the flag leaf of *T. dicoccum*. The range of SLW observed through successive leaves increased with ploidy within the *Triticum* species (Table 4 : 1, SLW).

A positive correlation between SLW and the photosynthesis

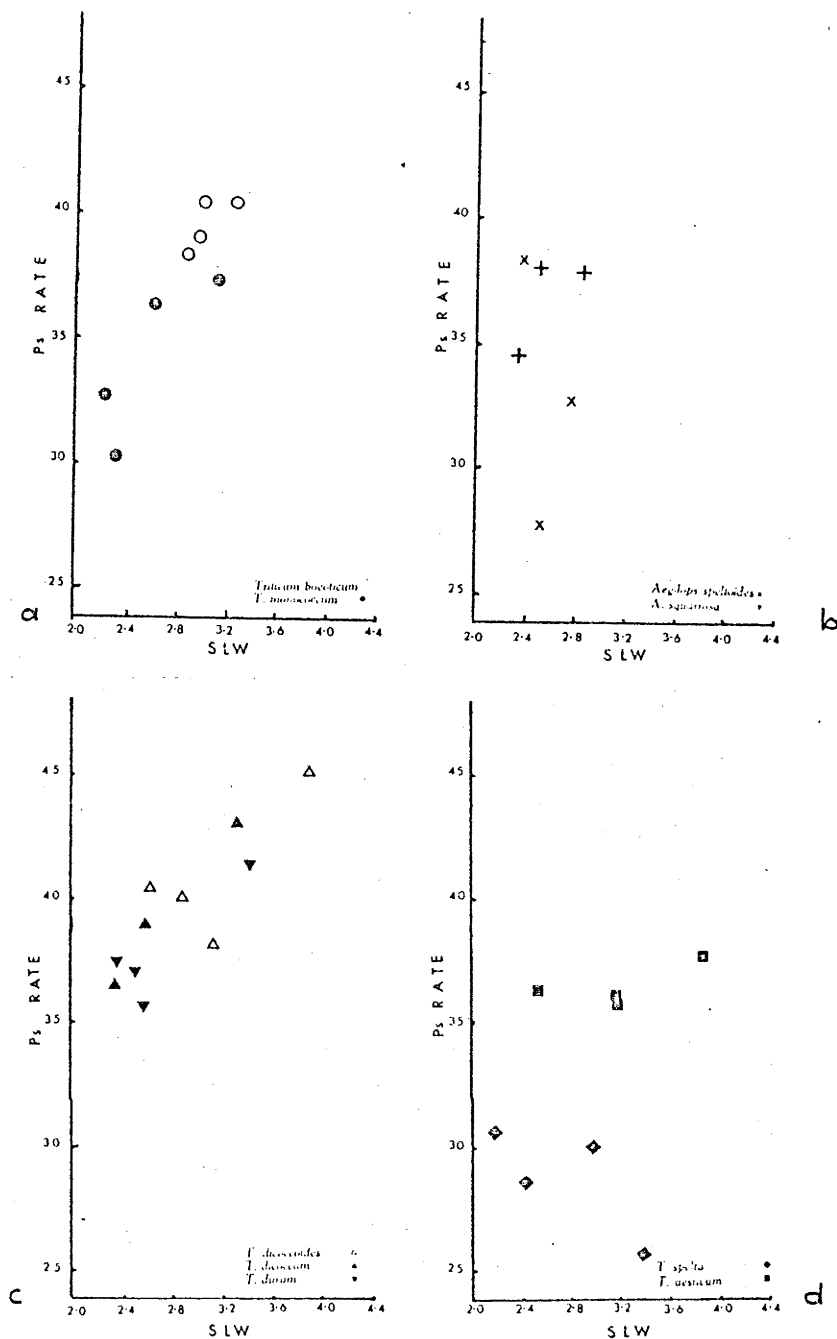


Figure 4 : 3

The relationship between the photosynthesis rate ($\text{mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$) at 7000 f.c. and atmospheric (CO_2) and the specific leaf weight (SLW) mg cm^{-2} . a. Diploid Triticum spp. $r = .88^{**}$ b. Aegilops spp. c. Tetraploid Triticum spp. $r = .85^{***}$ d. Hexaploid Triticum spp.

** $P < .01$ *** $P < .001$

rate held for all leaves of the diploid and tetraploid Triticum spp. but not for the hexaploid Triticum spp. or for the Aegilops spp. (Figure 4 : 3).

Stomatal Frequency

Stomatal frequency was consistently higher on the upper than on the lower surface of the leaves. The mean stomatal frequency of the upper and lower surface of each leaf is listed in Table 4 : 1, SF. The lowest mean frequency was for (F-3) of *T. aestivum* (3147 cm^{-2}) and the highest for the flag leaf of *T. monococcum* (7460 cm^{-2}).

The mean stomatal frequency rose from (F-3) to the flag leaf in all species.

A negative correlation was found between leaf area and stomatal frequency for the flag leaves only (Figure 4 : 4).

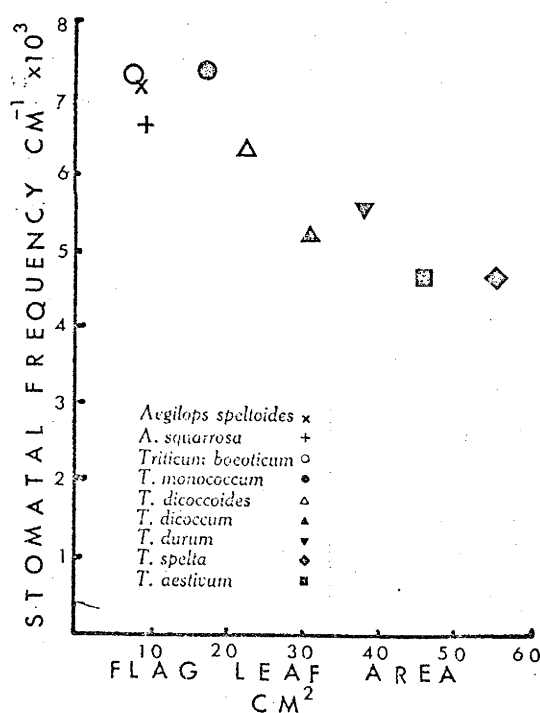


Figure 4 : 4

Relationship between the area of the flag leaf blade (cm^2) and the mean stomatal frequency cm^{-2} of the upper and lower surface of the flag leaf of each species. Each point is the mean of 4 fields of the upper and 4 fields of the lower surface of each of 4 leaves of each line of each species. $r = -.74$ $P < .01$.

Preliminary Discussion

The differences between the photosynthetic rates of the various species were greater for the flag leaves than for any of the earlier leaves measured.

The lowest range of rates measured in the present work was for (F-3) which was the fifth leaf developed and it is quite possible that with leaf 2 there are negligible differences between species as Belikov et al. (1961) have noted.

The data for the flag leaves in Experiment 2 are markedly different from those obtained for the same species in Experiment 1.

TABLE 4 : 2

A comparison of the photosynthesis data for the flag leaf blades of each species between Experiment 1 and Experiment 2

	<u>mg CO₂ dm⁻² hr⁻¹</u>	
	<u>E.1</u>	<u>E.2</u>
<u>T. boeoticum</u>	62.7	40.4
<u>T. monococcum</u>	48.3	37.1
<u>A. speltoides</u>	47.9	32.8
<u>A. squarrosa</u>	53.8	37.8
<u>T. dicoccoides</u>	46.6	45.5
<u>T. dicoccum</u>	43.0	43.0
<u>T. durum</u>	34.9	42.2
<u>T. aestivum</u>	36.9	37.8
<u>T. spelta</u>	32.0	25.7

All diploid species dropped in rate from Experiment 1 to Experiment 2. Tetraploid and hexaploid species did not change as markedly as the diploid spp. T. dicoccoides, T. dicoccum and T. aestivum remained stable but T. durum rose while T. spelta fell in Experiment 2.

It is clear that the changes which took place in photosynthesis rates between Experiments 1 and 2 were different for all species and a common pattern was not apparent.

Since the plants for both experiments were grown in a phytotron glasshouse, controlled for temperature and photoperiod, the only environmental variable which had obviously changed from Experiment 1 (a summer experiment) to Experiment 2 (a winter experiment) was the incident radiation during growth. Moreover it has been frequently reported in the literature that the rate of light-saturated photosynthesis is strongly influenced by the light intensity under which the plants are grown. (e.g. Hesketh, 1968; Woledge, 1971).

Friend (1966) infers a rise in photosynthesis with rise in light intensity, during the growth period for T. aestivum, basing his inference on the changes which took place in leaf thickness, cell size and chlorophyll content.

Hesketh (1968) found that the hexaploid T. aestivum, var. Gabo, gave the same rates when grown under winter and spring light, but decreased in rate when grown under the high light conditions of summer.

Of particular significance for the present work is the observation of Bjorkman and Holmgren (1963) of a strong adaptation to high light in ecotypes of several species from exposed habitats but practically none in ecotypes from shaded habitats.

The differences in absolute values of photosynthesis and in the ranking of the species between Experiment 1 and Experiment 2 would be consistent with a differential adaptation of the species to the light intensity under which the plants were grown. It is possible that the diploid Triticum and Aegilops species behave as "sun ecotypes" while the polyploid Triticums behave as Bjorkman and Holmgren's shade ecotypes.

In Experiment 2 the negative relationship found between photosynthesis rate and leaf area in Experiment 1 was not sustained (Figure 4 : 2). This appears to be due mainly to a large drop in the photosynthesis rate of the diploid species without a correspondingly large increase in leaf area.

A number of authors have noted a correlation between specific leaf weight (SLW) and light saturated photosynthesis in a variety of species (Cooper and Qualls, 1967; Pearce et al. 1969; Beuerlein and Pendleton, 1971). Such a relationship is clear only with the diploid and tetraploid Triticum species so it is doubtful if changes in specific leaf weight are very closely associated with the differences observed in photosynthesis rates.

No relationship was found between photosynthesis rate and stomatal frequency for either the flag leaves or the lower leaves. A similar result was found for Lolium perenne L. by Wilson and Cooper (1969).

The negative relationship found between stomatal frequency and leaf area (Figure 4 : 4) may well result from the fact that increase in leaf area of modern wheats is caused by an increase in cell size. An increase in epidermal cell size would bring about a wider spacing of stomata. We could then expect to find a correlation between stomatal frequency and photosynthesis only in

those conditions where we find a negative correlation between photosynthesis and leaf area.

EXPERIMENTS 3 and 4

Photosynthesis, natural light - summer and autumn conditions

Introduction

Experiments 1 and 2 demonstrated that both the absolute photosynthesis rates and the ranking of the various species in these rates were not constant when plants were grown under controlled temperature and photoperiod but at different radiation levels.

It seemed probable that the different responses observed among the species to the conditions imposed in Experiment 1 and 2 were due to a differential adaptation to the radiation level under which the plants were grown. The diploid species may have behaved as sun ecotypes while the higher ploidy species responded as shade ecotypes.

Two experiments, 3 and 4, were carried out to measure the response of flag leaves to two different natural light regimes when grown in the phytotron glasshouse at 21/16°C with a 16 hour photoperiod.

Results of Experiments 3 and 4 were to be used to provide photosynthesis data for leaves grown under radiation levels intermediate between the high summer values of Experiment 1 and the low winter values of Experiment 2. Data would then be available for a comparison of material grown under a wide range of light conditions from full summer to midwinter.

Materials and Methods

The 21 lines used in these experiments were the same as for Experiment 2 with the addition of a further line of T. boeoticum coded G31. Unfortunately all lines of T. boeoticum were lost in Experiment 3 when the temperature in the refrigerator rose for a period during the vernalisation treatment causing the plants to become etiolated.

Measurements began on 22nd January for Experiment 3 and 29th March for Experiment 4.

Photosynthesis measurements were made on the flag leaves at ear emergence as reported for Experiment 2. Specific leaf weight and stomatal frequency were measured as before.

RESULTS

The net photosynthesis rates, leaf areas, specific leaf weights and stomatal frequencies obtained are shown in Table 5 : 1.

In Experiment 3 the photosynthesis rates ranged from 48.8 mg CO₂ dm⁻² hr⁻¹ for T. durum to 34.1 mg CO₂ dm⁻² hr⁻¹ for T. spelta (T. boeoticum not being represented).

In Experiment 4 the range was only slightly less than for Experiment 3, but the absolute values in all cases excepting Ae. squarrosa and T. durum had increased. The highest value was 53.7 mg CO₂ dm⁻² hr⁻¹ for Ae. speltoides and the lowest 38.4 mg CO₂ dm⁻² hr⁻¹ for T. spelta.

The leaf areas for Experiment 3 ranged from the small blades of Ae. speltoides, 6.7 cm², up to the large leaves of T. spelta, 46.5 cm². Leaf areas for Experiment 4 were larger than in Experiment 3 for all species excepting T. aestivum, but the range of areas increased only marginally, extending from T. boeoticum, 6.9 cm² to T. spelta, 49.9 cm².

A negative correlation was found between the net photosynthesis rate and the area of the flag leaf blade.

The increase in leaf area from Experiment 3 to Experiment 4 was accompanied by a decrease in specific leaf weight in all species except T. durum. Specific leaf weight (SLW) ranged from 3.46 mg cm⁻² for Ae. speltoides to 5.62 mg cm⁻² for T. dicoccoides in Experiment 3. There was no correlation between photosynthetic rate and SLW.

Table 5 : 1

Photosynthesis rates, flag leaf areas, specific leaf weights and stomatal frequencies from Experiments 3 and 4

	Ps Rate		Flag Leaf		SLW		Stomatal	
	mg CO ₂ dm ⁻² hr ⁻¹	Exp	Area	cm ²	mg	cm ⁻²	Frequency	cm ⁻²
	Exp	Exp	Exp	Exp	Exp	Exp	Exp	Exp
	3	4	3	4	3	4	3	4
<u>T. boeoticum</u>	-	50.9	-	6.9	-	4.15	-	8175
<u>T. monococcum</u>	45.4	46.9	9.6	16.0	4.41	3.28	8157	7601
<u>Ae. speltoides</u>	48.2	53.7	6.7	7.0	3.46	3.00	6540	7136
<u>Ae. squarrosa</u>	47.7	41.8	8.0	13.1	4.25	3.92	7391	7748
<u>T. dicoccoides</u>	47.0	48.2	20.6	32.5	5.62	4.87	-	6129
<u>T. dicoccum</u>	43.5	43.9	25.8	30.5	4.13	4.08	5580	5763
<u>T. durum</u>	48.8	44.7	35.2	39.2	3.90	4.16	5690	6203
<u>T. spelta</u>	34.1	38.4	46.5	49.9	4.61	4.26	5086	4995
<u>T. aestivum</u>	41.2	46.7	28.7	26.1	4.56	3.96	5574	4856

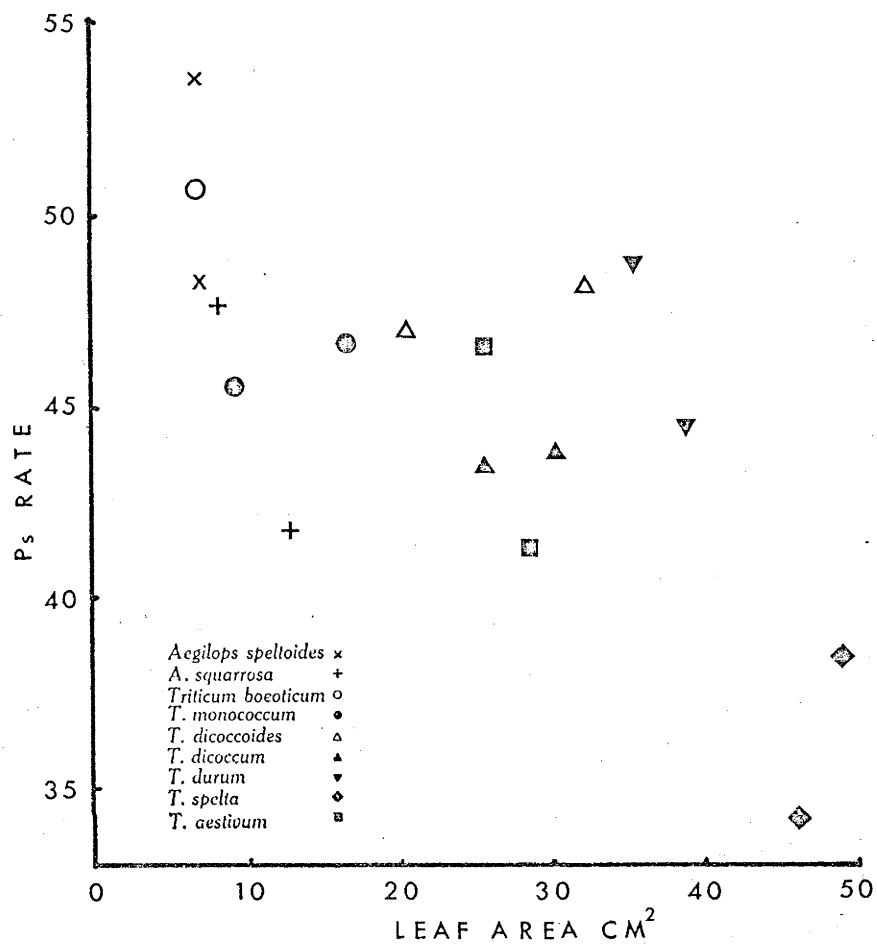


Figure 5 : 1

Relationship between the net photosynthesis rate at 7000 f.c. and atmospheric (CO_2) ($mg\ CO_2\ dm^{-2}\ hr^{-1}$) and the area of the flag leaf blade (cm^2). Each point is the mean for one species for one experiment. Data from Experiment 3 and 4 is included. $r = -0.64$
 $P < .01$.

In both experiments the stomatal frequency decreased with increase in ploidy. In Experiment 3 the stomatal frequency was greatest for T. monococcum and least for T. spelta. In Experiment 4 the stomatal frequency ranged from a maximum of 8175 cm^{-2} in T. boeoticum to a minimum of 4856 cm^{-2} in T. aestivum.

A weak correlation between stomatal frequency of photosynthetic rate was noted (Figure 5 : 2), but this may well be associated with a stronger correlation between leaf area and stomatal frequency (Figure 5 : 3).

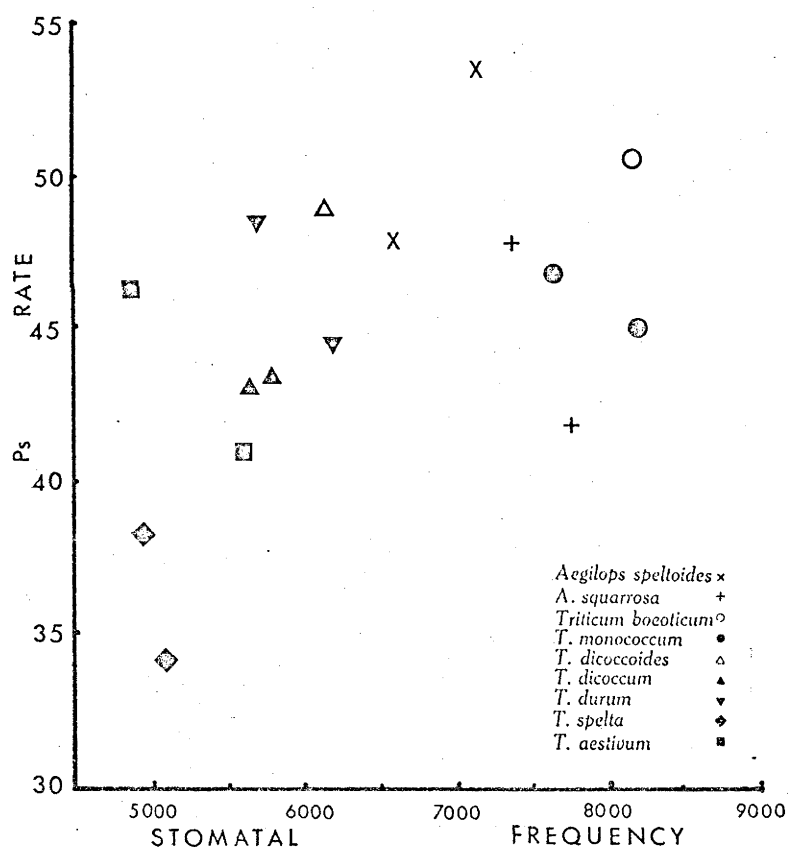


Figure 5 : 2

Relationship between the net photosynthesis rate of the flag leaf blade ($\text{mg CO}_2\text{ dm}^{-2}\text{ hr}^{-1}$) at 7000 f.c. and atmospheric (CO_2) and the mean stomatal frequency (cm^{-2}) of the upper and lower surface of the leaf. Each point is the mean for one species for one experiment. Data from Experiments 3 and 4 are included. $r = .52$. $P < .05$.

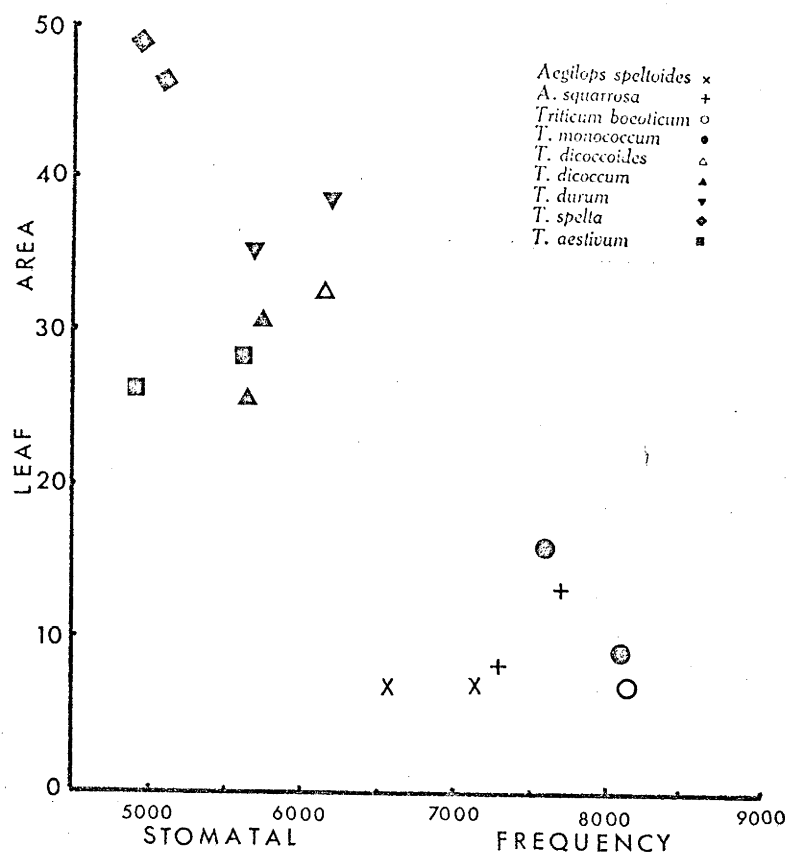


Figure 5 : 3

Relationship between the area of the flag leaf blade (cm²) and the mean stomatal frequency (cm⁻²) of its upper and lower surfaces. Each point is the mean for one species for one experiment. Data from Experiments 3 and 4 are included. $r = -.81$ $P < .001$.

Preliminary Discussion

In Experiment 1 there was a negative correlation between photosynthesis rate and leaf area. In Experiment 2 this correlation no longer held because the photosynthesis rates of the diploid species were severely depressed without there being a corresponding increase in leaf area. In Experiments 3 and 4 the rates of photosynthesis of the diploid species were higher than

in the midwinter experiment (2) and the negative correlation was once more apparent (Figure 5 : 1).

An examination of the data for Experiments 1 to 4 shows that, for plants grown under controlled temperature and daylength conditions but with varying intensities of natural light, the diploid lines had a greater range of photosynthesis rates than had the wheats of the other two ploidy groups.

EXPERIMENT 5

Photosynthesis, artificial light

Introduction

The variations in photosynthesis data for Experiments 1, 2 and 3 and 4 can be explained on the basis of a differential adaptation to the incident radiation during the growth period.

Under glasshouse conditions, the period of natural light varies according to the season. In winter time a longer period of low intensity photoperiod light is necessary to extend the natural light period to achieve a daylength of 16 hours, and this longer period of low light, in winter as compared with summer, could be responsible for the change observed in photosynthetic response of the leaf to light as measured in the leaf chamber.

To clarify this point and to investigate more closely the adaptation of both primitive and modern species to different light intensities during the growth period, an experiment was planned using closely controlled light conditions with a constant intensity for the 16 hour light period.

Materials and Methods

Four lines were chosen for the experiment. Two lines were varieties of T. boeoticum, lines T6625 and C64-146, which appeared to have a high level of adaptation to light intensity in the natural light experiments. Two lines were of hexaploid species; T. spelta (H2) and T. aestivum (Cappelle Desprez), which appeared to have a lower level of adaptation in the natural light experiments.

The seed was imbibed and vernalised in the refrigerator for eight weeks. The seeds were then planted out in a 21/16°C glasshouse and allowed to remain there to establish for 9 days.

The plants were then separated into two random groups

and placed in an artificially lit LB type cabinet (Morse and Evans, 1962). The LB cabinet had, in addition to the standard 28.140w fluorescent lamps, two Philips HPLR 1000w high pressure mercury vapour lamps.

One half of the cabinet was shaded with 2 layers of grey "Sarlon" shade cloth. The light intensity in the high light side of the cabinet was 4600 f.c., $174,800 \text{ ergs cm}^{-2} \text{ sec}^{-1}$ visible (HL pretreatment) while the shade side had an intensity of 2200 f.c. $83,600 \text{ ergs cm}^{-2} \text{ sec}^{-1}$ visible (LL pretreatment) at plant height.

At ear emergence the photosynthesis rates of the flag leaves of the plants were measured in groups of 3 (or sometimes 2) in the assimilation chamber.

The maximum light intensity used was 8600 f.c. Lower light intensities of 5200 f.c., 3600 f.c., 2800 f.c. and 1600 f.c. were achieved by placing 1, 2, 3 and 5 layers of white organdy fabric over the leaf chamber.

Leaf area was measured and leaf surface impressions taken as previously to record stomatal frequency. A measured section was taken from the centre of the leaf and fixed in FAA for later sectioning, while the remainder of the leaf was dried so that SLW could be calculated.

RESULTS

The photosynthesis rates, measured at 8600 f.c. ranged from $51.6 \text{ mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$ for the high light (HL) pretreatment of C 64-146 to $31.0 \text{ mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$ for the low light (LL) pretreatment of T-6625.

Light response curves are shown for each pretreatment of each species in Figure 6 : 1. The diploid lines T-6625 and C 64-146 had maximum rates of photosynthesis for their HL pretreatments

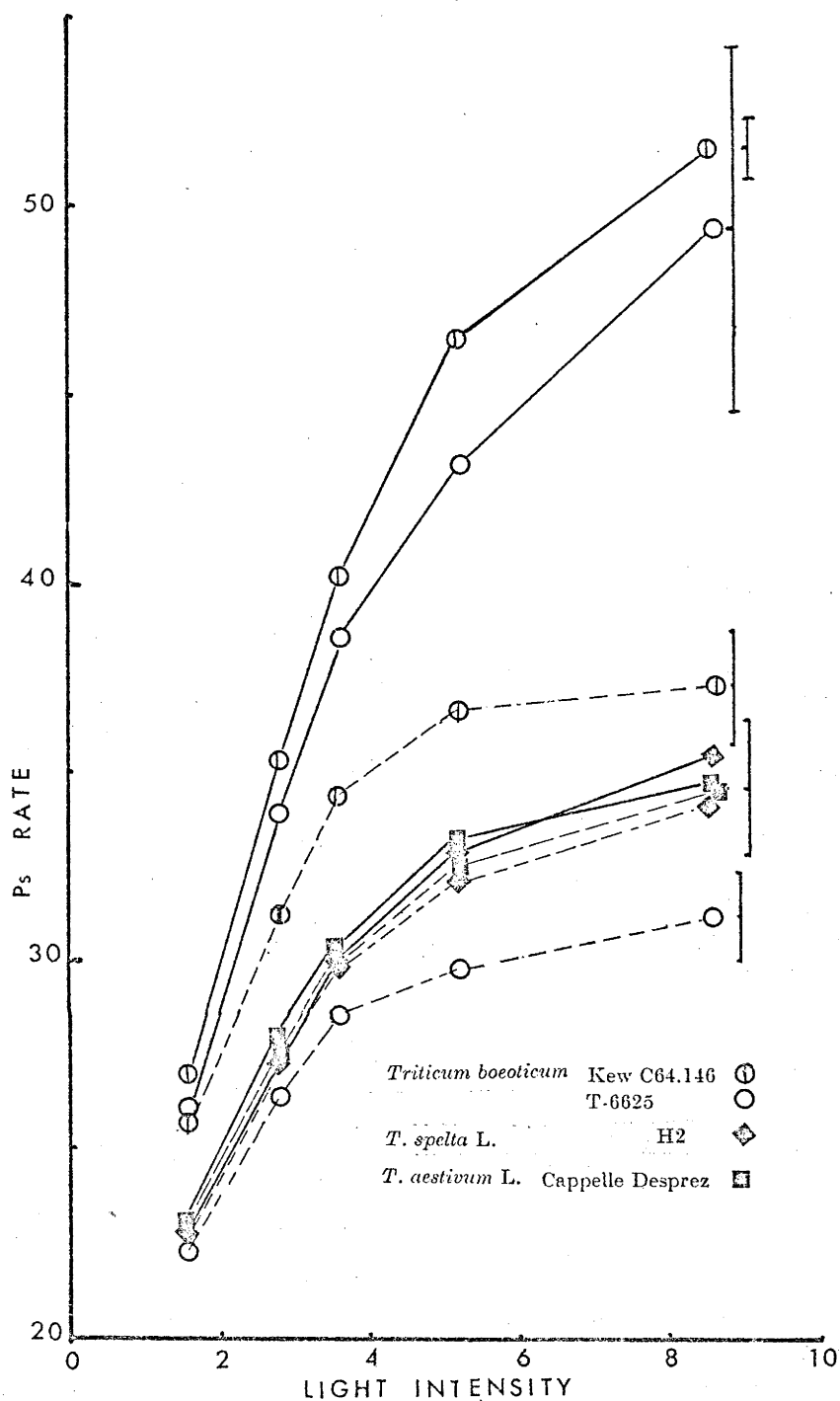


Figure 6 : 1

Graph showing the response of net photosynthesis of the flag leaf ($\text{mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$) to light intensity ($\text{f.c.} \times 10^{-3}$) for the HL pretreatments (—) and LL pretreatment (----) of each species. The vertical bars represent $2 \times \text{SE}$.

which were 50 to 60% higher than the corresponding LL pretreatments. The hexaploid lines did not have the same degree of response to pretreatment, and the photosynthesis rate of the HL pretreatment was almost identical to that of the LL pretreatment.

The HL pretreatments of T6625 and C64-146 gave steeper response curves and less evidence of light saturation at 8600 f.c. than the LL pretreatments or either pretreatment of the hexaploid lines.

In all lines except T6625, the area of the flag leaf blade was greater for the LL pretreatment than the HL pretreatment (Table 6 : 1). This represented an increase of about 10% in C64-146 and Cappelle Desprez, and a much greater increase of 63.6% in H2.

The highest specific leaf weights were found in the HL pretreatments of the hexaploid lines and the lowest in the LL pretreatments of the diploid lines (Table 6 : 1). There was a correlation of $r = .96$ ($P < .001$) between net photosynthesis rate and SLW for the diploid lines only (Figure 6 : 2).

Leaf thickness for HL pretreatments were as great as, or greater than for the LL pretreatments for every line. Photosynthesis tended to increase with leaf thickness up to 150 μ after which the trend was no longer clear (Figure 6 : 3).

Table 6 : 1

Data for the high light (HL) and low light (LL) pretreatments of the four lines used in Experiment 5

	High Light						Low Light					
	Ps	LA	PsF	LT	SLW	SF	Ps	LA	PsF	LT	SLW	SF
T-6625	49.5	7.79	3.86	172	4.33	U.9535 L.9272	31.0	6.1	1.89	110	3.10	U.8708 L.8525
C64-146	51.6	7.31	3.79	158	4.20	U.10,337 L.10,468	34.3	8.1	2.78	152	3.53	U.8562 L.9111
H2	36.2	42.8	15.49	162	5.81	U.6268 L.5093	34.5	70.0	24.15	145	4.12	U.5254 L.4127
Cappelle Desprez	36.4	59.6	21.69	152	5.50	U.6586 L.4464	35.0	65.5	22.93	151	4.20	U.6476 L.4464

Ps Photosynthesis rate of the flag leaf blade at 8600 f.c., mg CO₂ dm⁻² hr⁻¹.
 LA Area of the flag leaf blade, cm².
 PsF Photosynthesis rate of the total flag leaf blade, mg CO₂ flag⁻¹ hr⁻¹.
 LT Leaf thickness, μ.
 SLW Specific leaf weight, mg cm⁻².
 SF Stomatal frequency, Number cm⁻².

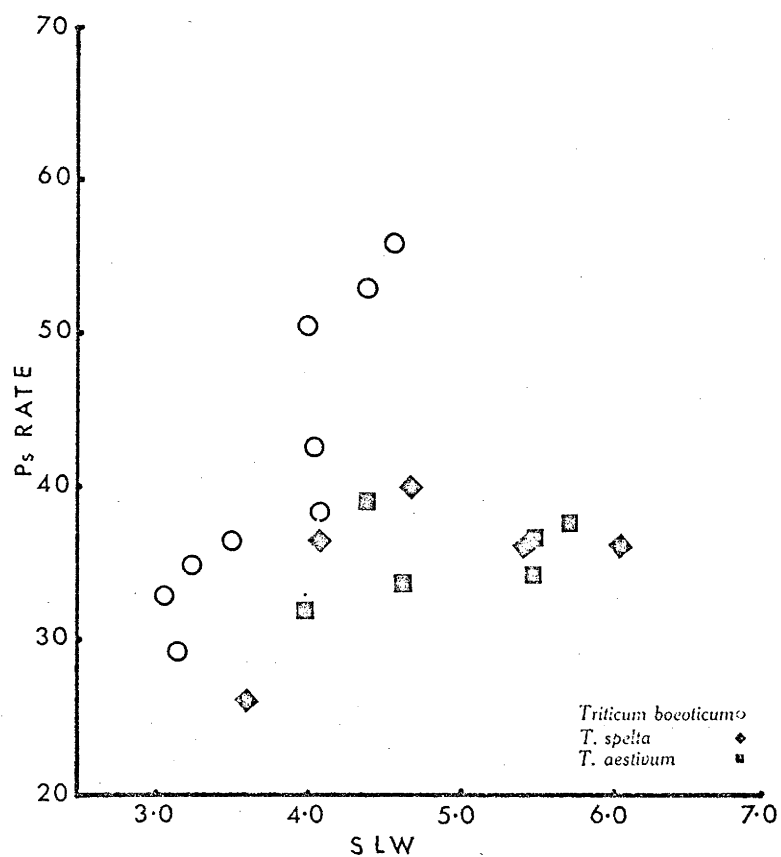


Figure 6 : 2

Relation between net photosynthesis (Ps) rate of the flag leaf blade at 8600 f.c. and atmospheric (CO₂) (mg CO₂ dm⁻² hr⁻¹) and specific leaf weight (SLW) (mg cm⁻²). Each point is the mean value per leaf obtained from one replicate of three leaves.

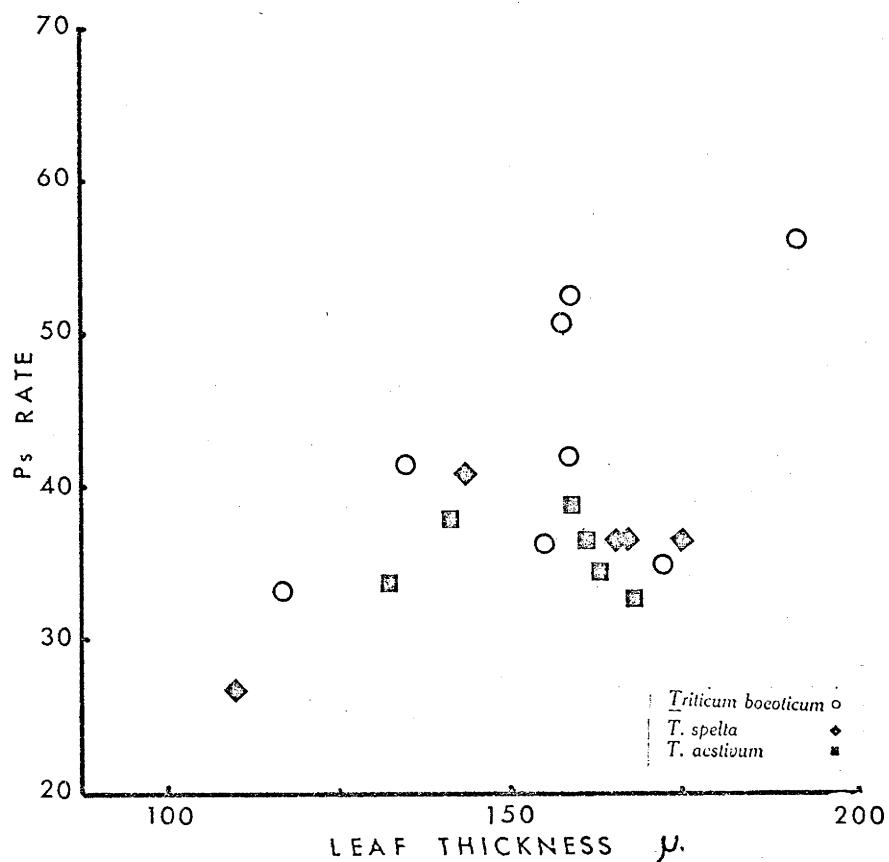


Figure 6 : 3

The relation between net photosynthesis (Ps) of the flag leaf blade at 8600 f.c. and atmospheric (CO_2) ($\text{mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$) and leaf thickness (μ). Each point is the mean value per leaf obtained from one replicate of three leaves.

Stomatal frequency was greater for the diploid lines than the hexaploid lines (Figure 6 : 4).

In all lines, excepting C64-146, the stomatal frequency on the upper surface was greater than the frequency on the lower surface. The HL pretreatment had a higher frequency than the LL pretreatment for all lines excepting Cappelle Desprez where there was no change.

The total photosynthesis per flag leaf blade was greater for both pretreatments of the hexaploid lines than for either pretreatment of the diploid lines (Figure 6 : 5).

For the diploid lines, the HL pretreatment had a greater rate of photosynthesis per flag leaf than the LL pretreatment.

For the hexaploids, the LL pretreatment had a higher value than the HL pretreatment.

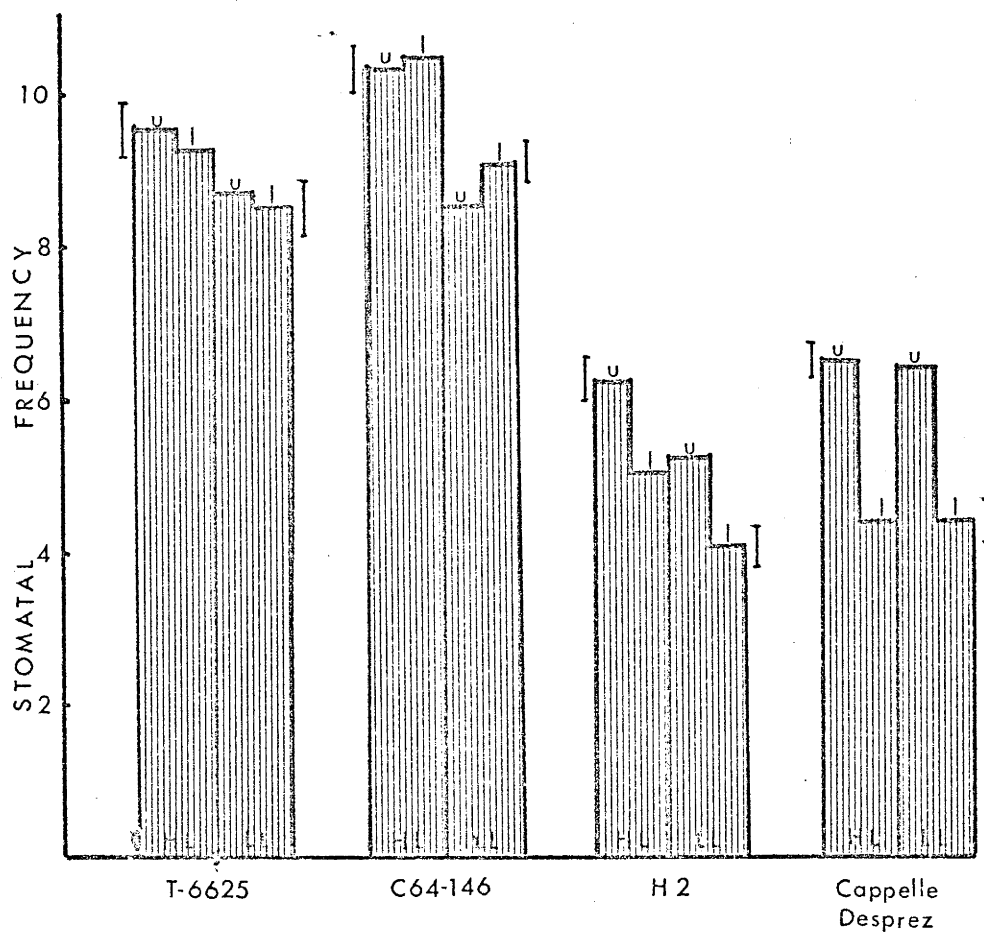


Figure 6 : 4

Histogram showing the stomatal frequency ($\text{No. cm}^{-2} \times 10^{-3}$) for the upper surface (U) and the lower surface (L) of the high light (HL) and low light (LL) pretreatment of each of four lines. The vertical bars represent $2 \times \text{S.E.}$ for the mean value of U and L.

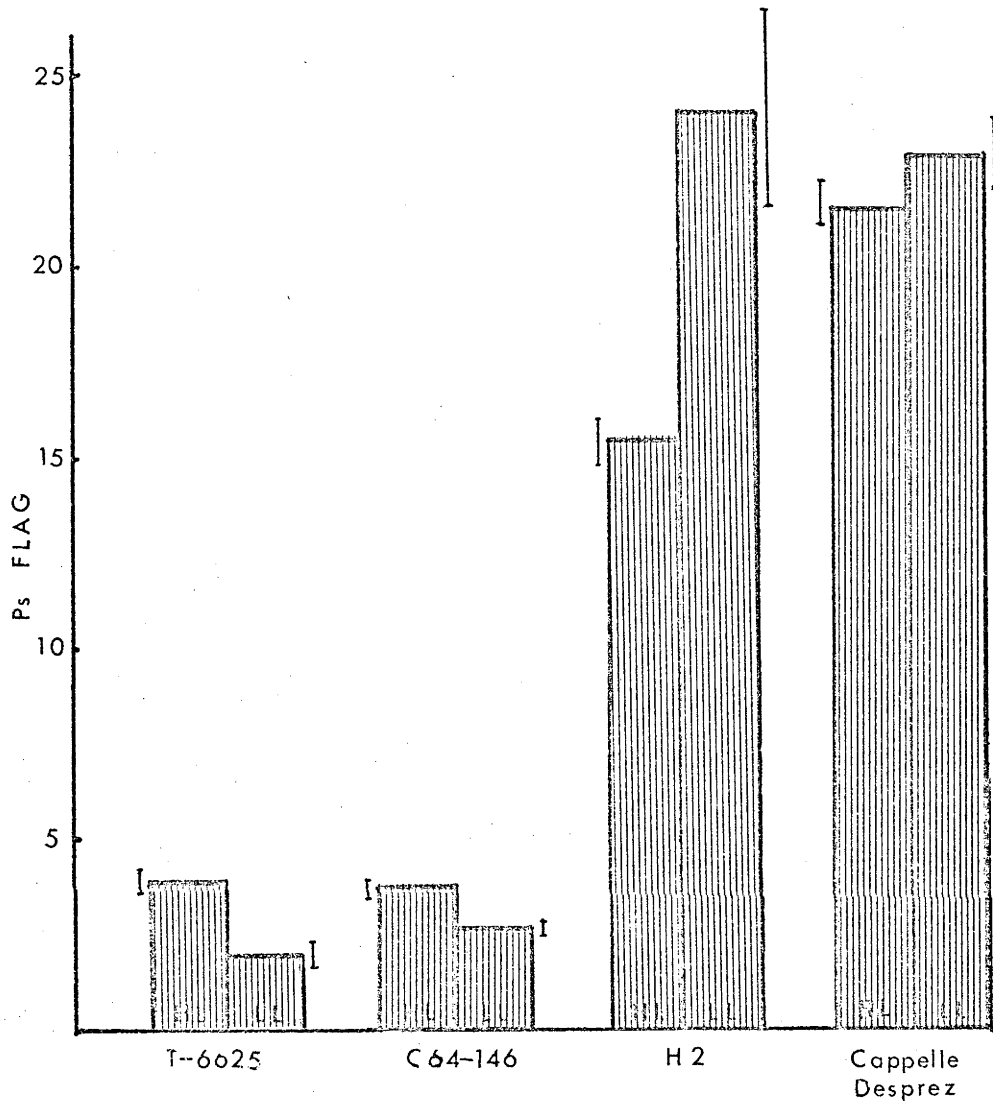


Figure 6 : 5

Histogram showing the total flag leaf photosynthesis (mg CO₂ flag hr⁻¹) for the high light (HL) and low light (LL) pretreatments of each line. The vertical bars represent 2 x S.E.

Preliminary Discussion

High light pretreatment resulted in marked changes in the maximum rates of photosynthesis in the diploid species but not in the hexaploid species.

The result is similar to that obtained in the experiments grown under natural light where there was a large difference between the maximum rates of photosynthesis for the summer and winter experiments in the diploid lines but only small differences for hexaploid lines.

This experiment suggests that the changes observed within each line in the maximum photosynthesis rate between experiments carried out in natural light were due to adaptation to the light intensity under which the plant was grown, and that the degree of adaptation to light intensity has changed with evolution in wheat.

EXPERIMENT 6

The Role of Cell Size Changes in the Evolution of Wheat

Introduction

The parallel increase with evolution in grain size and leaf size reported in Experiment 1 suggested an experiment to investigate changes in cell size as a possible basis for the increase in both organs. Schwanitz (1966, page 18) reported an increase in the size of the cells on the surface of the glumes of wheat both with increase in ploidy from diploid to tetraploid and with selection at those ploidies. The most likely common basis for a parallel increase in grain size and leaf size seemed to be a parallel increase in endosperm and mesophyll cell size.

Moreover, under the summer conditions of Experiment 1, the photosynthesis rate per unit area was found to fall with evolution. Thus an increase in mesophyll cell size (with evolution) in wheat could well be associated with a decrease in photosynthesis rate per unit area as was found for Lolium perenne by Wilson and Cooper (1967).

Materials and Methods

The lines used were those listed in Table 2 : 1 with lines G31, 6001, G90, T-6252 and W2698 omitted. The seeds were vernalized in the refrigerator and grown under the natural light conditions. The mean radiation level received from two weeks before the first harvest was $558 \text{ cal cm}^{-2} \text{ day}^{-1}$ total and $611 \text{ cal cm}^{-2} \text{ day}^{-1}$ during the period of grain filling.

The leaf below the flag leaf (F-1) was chosen as a representative leaf for the plant because it developed late on the plant but was unlikely to compete with the developing ear during its growth. The leaf blade was considered mature when it had fully emerged and the flag leaf had more than half emerged.

At this stage the photosynthesis rate of the leaf was measured at 8600 f.c. ($326,800 \text{ ergs cm}^{-2} \text{ sec}^{-1}$ visible). Measurements were made

on 3 lots of 3 leaves for each line. After measurement these leaves were fixed for sectioning and cell separation. A further nine plants were left in the glasshouse until the grain matured. Selected grains were then used for weight and volume measurements and then sectioned.

RESULTS

Leaf Structure

An examination of the cross-sections of the leaves showed that the diploid species had mesophyll cells which were arranged loosely in a radiate pattern.

The tetraploid and hexaploid lines had mesophyll cells which did not have the radiate pattern and which were more tightly packed. A similar pattern was noted by Khan and Tsunoda (1970d), but in their samples the leaves of the diploid lines were more tightly packed than those of the tetraploid and hexaploid lines.

The projected area of separated mesophyll cells of the leaves of the cultivated tetraploid and hexaploid lines were 1.5 to 2 times larger than the diploids. However the mesophyll cell size of the wild tetraploid T. dicoccoides was within the range of the diploid lines (Table 7 : 1, column 3).

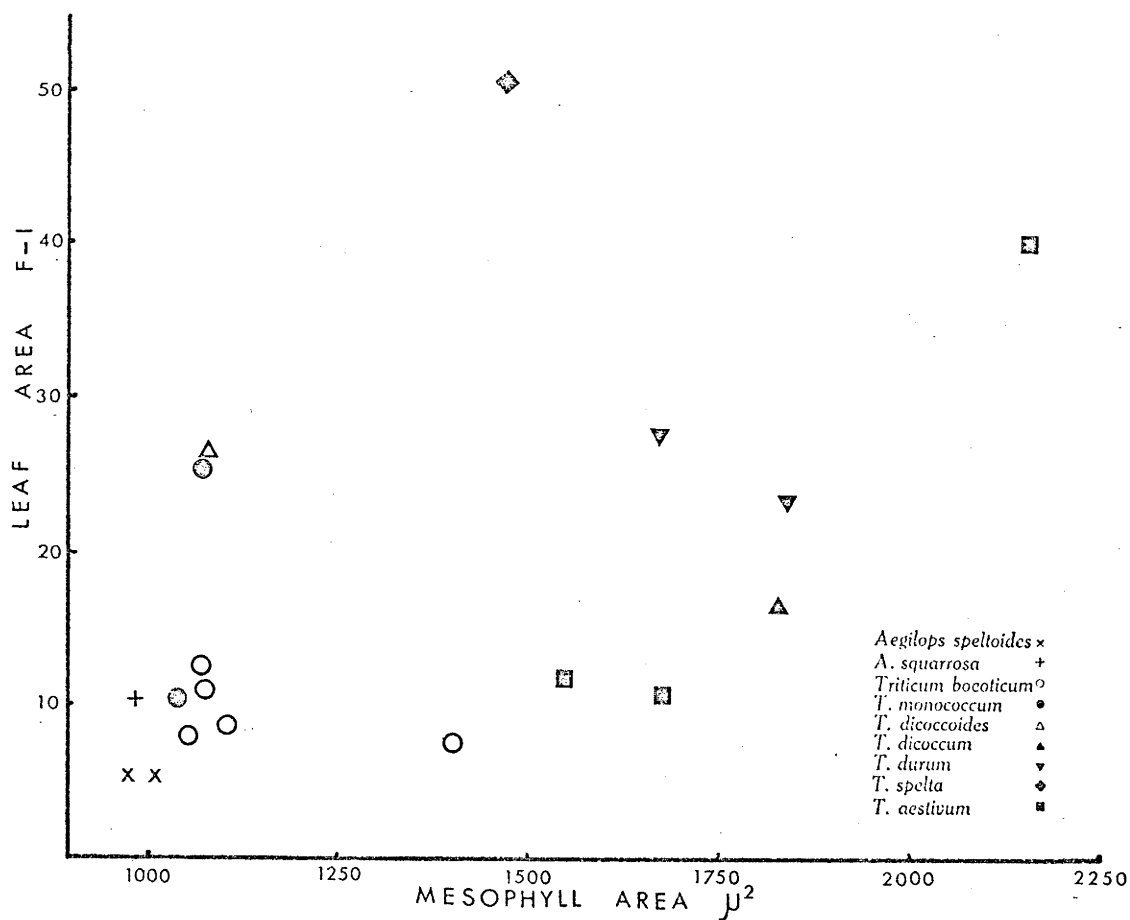
There was no change in mesophyll cell size with the shift from wild to cultivated at the diploid level, but there was an increase of up to 69% with this shift at the tetraploid level.

There was a correlation between the projected area of the mesophyll cells and the area of the corresponding leaf (Figure 7 : 1).

There was a negative correlation between the projected area of the mesophyll cells and the rate of net photosynthesis of the penultimate leaf. It is notable that the Aegilops species fell below the line common to other species on this graph (Figure 7 : 2).

TABLE 7 : 1

	Photosynthetic rate of (F-1) at 8600 f.c. mg CO ₂ dm ⁻² hr ⁻¹	Leaf Area (F-1) cm ⁻²	Mean Projected Area of Mesophyll cells μ^2	Mean Grain wt. Basal grains 4 central spk. mg	Mean Grain vol. Basal grains 4 central spk. mm ³	Cross sectional Area of endosperm cells μ^2	% Nitrogen grain
<u>T. boeoticum</u>	51.4	9.7	1144	16.8	14.5	4846	4.96
<u>T. monococcum</u>	47.5	18.4	1058	34.1	26.5	4865	4.42
<u>A. speltoides</u>	32.9	5.3	989	23.3	20.0	4790	3.29
<u>A. squarrosa</u>	19.9	12.1	941	8.1	8.7	4770	4.46
<u>T. dicoccoides</u>	47.2	26.3	1092	41.6	34.2	6160	4.37
<u>T. dicoccum</u>	46.8	16.8	1843	66.2	50.6	5690	3.42
<u>T. durum</u>	44.3	25.4	1754	61.2	50.8	5015	3.18
<u>T. aestivum</u>	41.3	21.0	1783	59.0	47.6	5087	3.14
<u>T. spelta</u>	39.3	50.9	1467	64.3	54.5	5450	-



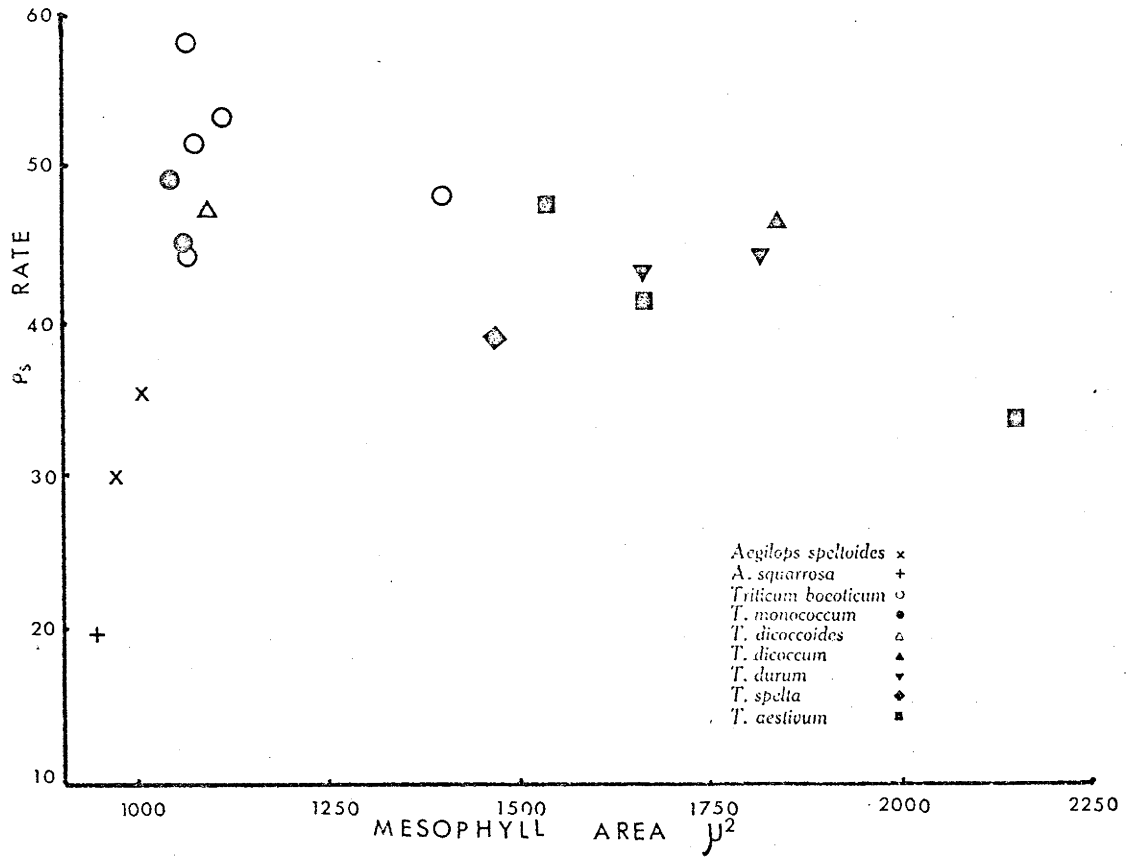


Figure 7 : 2

Relation between the net photosynthesis rate of the F-1 leaf ($\text{mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$) of each line measured at 8600 f.c. and atmospheric (CO_2) and the mean projected area of the mesophyll cells (μ^2) of that leaf. $r = -0.86$ $P < .001$.

The mean grain weight for the basal grains of the four central spikelets (Table 7 : 1, column 4) increased with increase in ploidy from the diploid to the tetraploid stage but there was no further increase in grain weight with increase in ploidy to the hexaploid stage. Grain weight increased with selection from wild to cultivated at both the diploid and tetraploid level.

A strong correlation between weight per grain and grain volume (Figure 7 : 3) indicated that there was no change in grain density with increase in grain weight.

The cross sectional area of the endosperm cells was greater for the tetraploid and hexaploid lines than for the diploid lines (Table 7 : 1, column 6). There was no simple relation between the cross sectional area of the endosperm cells and the grain volume (Figure 7 : 4).

Although both mesophyll and endosperm cell sizes were smaller for the diploid lines than for the tetraploid and hexaploid lines there was no evidence for a correlation between the two (Figure 7 : 5).

The percentage nitrogen in the grain decreased with evolution from 4.96% for T. boeoticum to 3.14% for T. aestivum (Table 7 : 1, column 7). This decrease in percentage nitrogen was associated with the increase in grain weight with evolution (Figure 7 : 6).

An examination of the slides stained with Ponceau 2R protein stain indicated that all grains had a high concentration of protein bodies in the aleurone and sub-aleurone layers. The large grains of the cultivated tetraploid and hexaploid lines had large areas in the centre of the lobes of the grain which were tightly packed with starch grains but apparently low in protein.

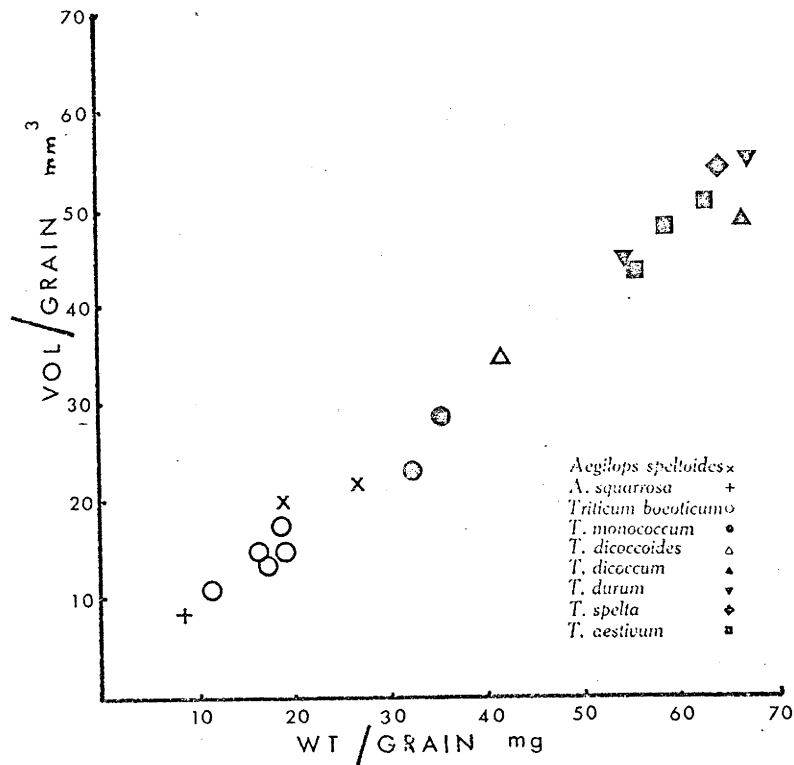


Figure 7 : 3

Relation between the mean weight per grain (mg) of the basal grains from the central 4 spikelets of the mainstem ear of each line and the grain volume, (mm³). $r = 0.99$ $P < .001$.

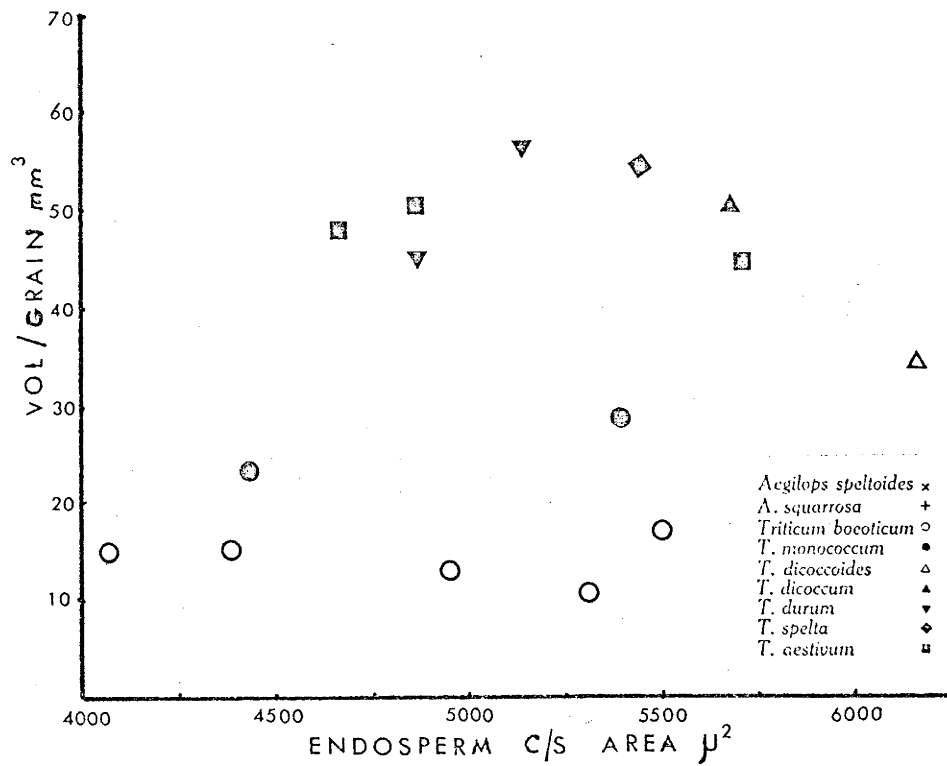


Figure 7 : 4

Relation between the mean cross-sectional area of the endosperm cells (μ^2) and the mean volume (mm^3) of the basal grains of the central 4 spikelets of the mainstem ear of each line.

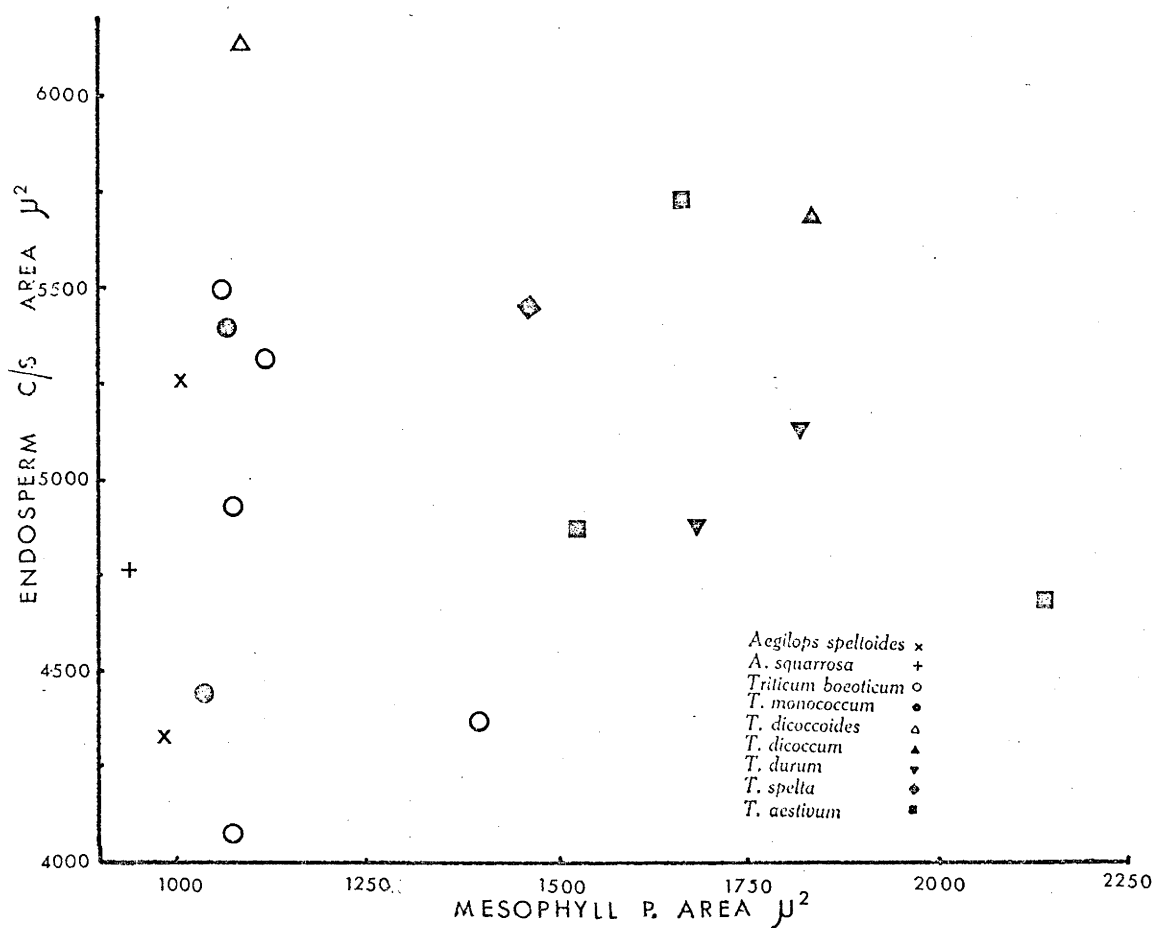


Figure 7 : 5

Relation between the mean cross-sectional area of the endosperm cells (μ^2) of the basal grains of the central 4 spikelets of the mainstem ear, and the projected area of the mesophyll cells (μ^2) of leaf F-1 of each line.

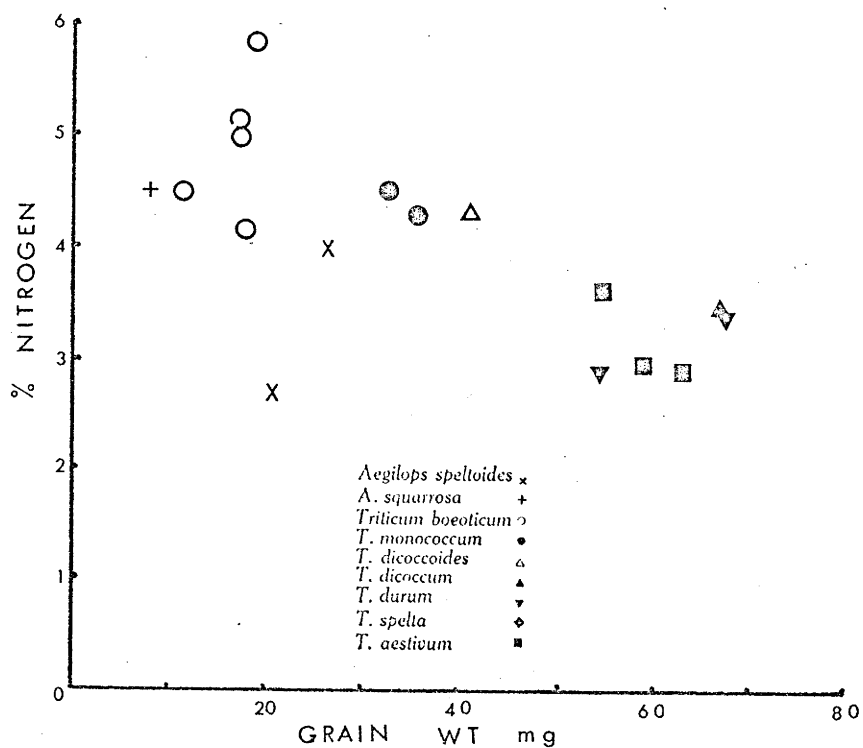


Figure 7 : 6

Relation between the grain weight (mg) and the percentage nitrogen in the grain. $r = -0.64$ $P < .01$.

GENERAL DISCUSSION

Grain and Leaf Growth

Increase in grain size accompanied by a parallel increase in leaf area has been the outstanding feature of the evolution of wheat. The great range in leaf and grain size, and the close relation between them was illustrated in Figure 3 : 3. There is evidence that grain size has been an important criterion for selection at least during the early stages of wheat evolution. The much larger grains of T. monococcum compared with those of the wild diploid T. boeoticum (Table 3 : 1) support this conjecture. It has been observed that the Neolithic grains of T. monococcum (Percival 1921) and T. dicoccum (Helbaek, 1966) were smaller and more closely resembled their wild progenitors than do modern cultivars. Several early writers on agriculture, such as Columella (Res Rustica 2, 9, 11), Varro (Res Rusticae 1, 52) and Virgil (Georgics 1, 197) emphasised the importance of selecting the largest grains of the harvest for the next sowing, a practice which may be as old as agriculture itself.

The problem then remains to examine changes in anatomical features or physiological processes which may have been associated with the increase in grain size with evolution.

Physical Restrictions

It is possible that the grain size especially in the primitive invested grain wheats is restricted in size by the enclosing glumes as has been reported for rice, Matsushima (1966, p.266). Bosknakian (1918) has discussed evidence for the mechanical restriction by the glumes of grain growth in wheat, such as the parallel marks of the glume veins along the mature grains. These are most evident in species with invested grains such as T. monococcum and T. spelta. However, the dimensions of glumes and of grains have probably changed

in parallel (cf. Lamba, 1949), with the result that glume size may not greatly limit grain size. In Experiment 1 the invested grains of T. spelta were amongst the largest, in spite of their investing glumes (Table 3 : 1).

The Role of Cell Size Increases

The possibility that the parallel increase in leaf and grain size was associated with a general increase in cell size was investigated in Experiment 6. Schwanitz (1966, p.17) was of the opinion that "As a rule, doubling of the chromosomes is accompanied by a doubling of cell volume". The results of the present work did not indicate that this conclusion can be applied to wheat either for the mesophyll cells or for the endosperm cells. With endosperm cells, the cross-sectional area increased with increase in ploidy from diploid to tetraploid but not from tetraploid to hexaploid (Table 7 : 1). The same applied to the mesophyll cells excepting that the projected area of the mesophyll cells of the tetraploid T. dicoccoides was no greater than for the diploid Triticum species.

Furthermore no consistent increase in cell size with selection for cultivation was found. The only case of increase in cell size with selection was found with mesophyll cells at the tetraploid level. This is remarkable since Schwanitz (1966, p.18) found such an increase in the size of the cells on the surface of glumes of wheat with selection at both the diploid and tetraploid levels.

The increase in mesophyll cell size could, at least in part, account for the increase in leaf area with evolution in wheat (Figure 7 : 1) but it is obvious that in some lines, such as H2 (T. spelta), an increase in cell numbers has also been important.

The increase in grain volume with evolution was not simply related to an increase in endosperm cell size (Figure 7 : 4) and it is not clear how the interaction between increases in cell size and those

in cell number has operated to increase grain volume with evolution.

Photosynthesis

An increase in the rate of photosynthesis per unit leaf area has not been a contributory factor to the increase in grain yield with evolution. When the plants were grown and measured under high light conditions, a drop in photosynthetic rate with evolution was observed (Experiment 1). A similar result was noted by Khan and Tsunoda (1970a). Since the photosynthetic rate has generally decreased as the grain yield has increased it seems unlikely that the supply of assimilate has been a limiting factor during evolution, although it is possible that it could limit further increase in grain size.

It is now clear that both the absolute values and the ranking of photosynthesis rates of leaves of different wheat and wheat progenitor species vary under different environmental conditions. Thus a comparison between the rates of net photosynthesis obtained by Khan and Tsunoda (1970a) and Evans and Dunstone (1970) although mutually confirmatory in the ranking of species, showed a wide difference in maximum rates. In contrast Belikov et al (1961) could demonstrate no differences between species at three levels of ploidy.

Differences in results obtained by the three groups of experimenters could be due primarily to differences in the environments within the leaf chambers during measurements. However, the differences observed between the photosynthesis rates of the early leaves of the various species were less than the differences between the flag leaves (Experiment 2). This may well be the reason that Belikov et al (1961) found no differences between species when they measured the rate of the second leaf developed.

In the present work different rates of photosynthesis and different rankings of the species were obtained with each experiment

when measurements were carried out on the flag leaves under identical conditions (apart from the lower flow rate used in Experiment 1).

It is proposed that the differences in rates and ranking of flag leaf photosynthesis observed between experiments was mainly due to a differential adaptation to the level of radiation under which the plants were grown prior to measurement.

Bjorkman and Holmgren (1963) reported a differential adaptation to the level of radiation during growth for clones of Solidago virgaurea from exposed and shaded habitats. Further, they found that the differences extended to other genera studied, including Rumex, Geum, Lamium and Plantago (Bjorkman and Holmgren, 1966). Their conclusion was that clones from exposed habitats had higher rates of light saturated photosynthesis when grown under high light conditions than those clones from shade habitats when grown under the same conditions. When grown under low light conditions, the clones from shaded habitats gave higher rates than those from exposed habitats.

Hesketh (1968), growing a range of species in the same phytotron glasshouses used in the present experiments found that the maximum rates of photosynthesis changed through midwinter, spring to midsummer experiments. Some species such as Zea mays and Helianthus annuus gave substantially higher rates in the midsummer experiment than the midwinter while T. aestivum showed equal rates for midwinter and spring and a depressed rate in midsummer.

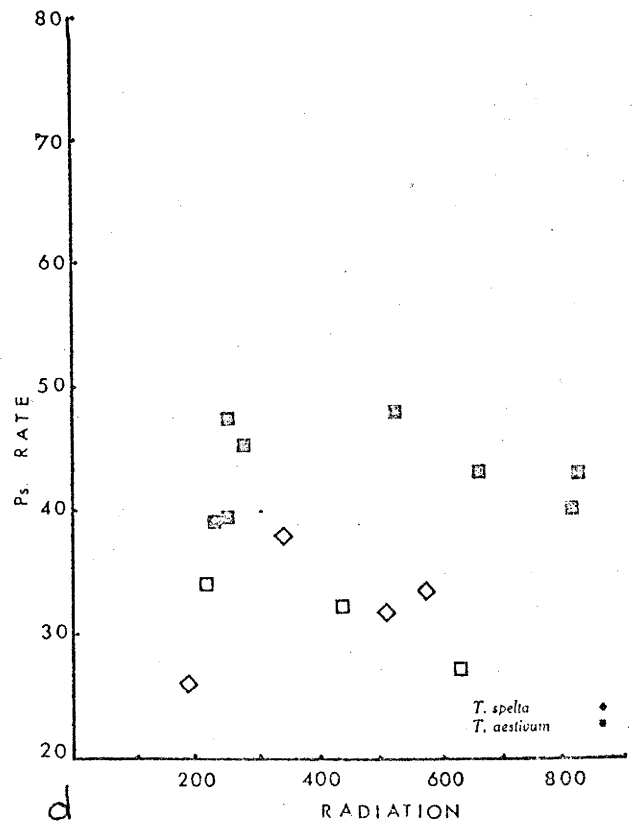
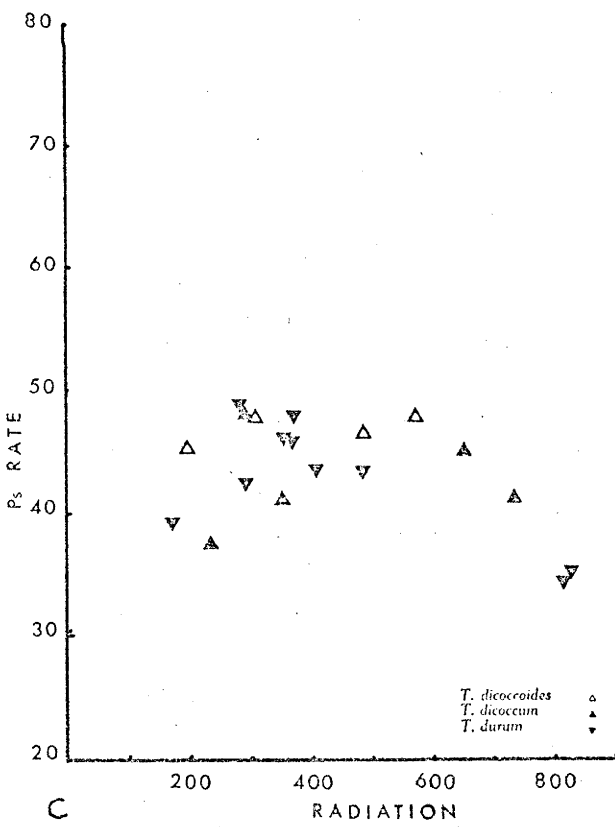
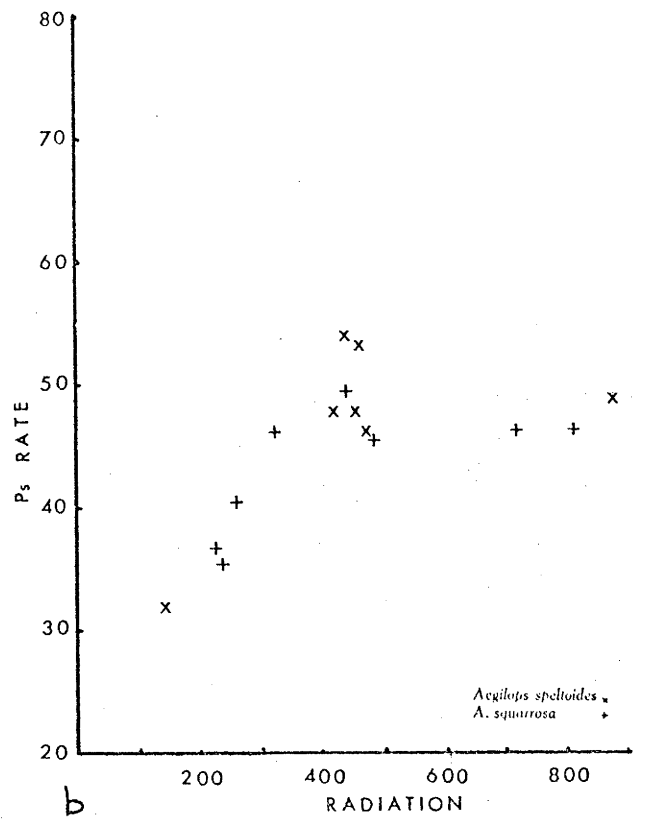
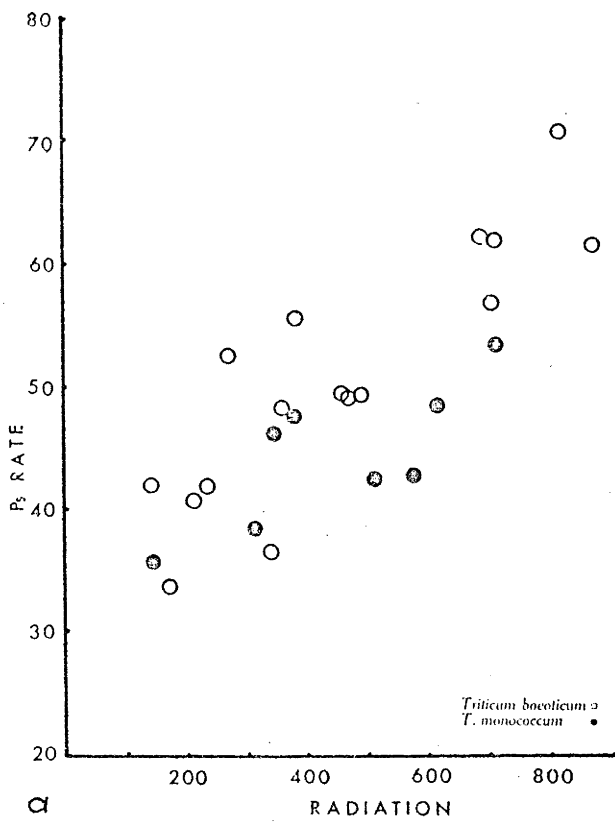
In Figure 8 : 1, the photosynthesis data for the flag leaf of each line for each of the experiments with glasshouse grown plants (Experiments 1 - 4) have been plotted against the mean incident radiation for the three days prior to measurement. It was decided to use the short period of three days before measurement because the most

recent light regime under which the plants are grown has been shown to have the greatest effect on the photosynthetic response of the leaves (Pearce and Lee, 1969).

Figure 8 : 1 (Overleaf)

Legend: Relationship between the photosynthesis rate of the flag leaf blade ($\text{mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$) at 7000 f.c. and atmospheric (CO_2), and the mean total daily radiation ($\text{cal cm}^{-2} \text{ day}^{-1}$) above the glasshouse for three days prior to measurement. Each point is the mean for one line for one experiment. Data from Experiments 1 to 4 are included.

- | | |
|------------------------------------|---|
| a. Diploid <u>Triticum</u> spp. | b. <u>Aegilops</u> spp. |
| c. Tetraploid <u>Triticum</u> spp. | d. Hexaploid spp. |
| | (Open symbols, winter varieties.
Closed " , spring varieties.) |



It is clear that the four groups, diploid Triticum spp., Aegilops spp., tetraploid Triticum spp. and hexaploid Triticum spp. were adapted in different ways to the light regime under which growth took place.

The diploid Triticum species show a strong correlation ($r = .80$) between the photosynthetic rate of the flag leaf blade at 7000 f.c. and the mean radiation received for three days prior to measurement. The relationship is maintained up to the highest radiation figures obtained (over $800 \text{ cal cm}^{-2} \text{ day}^{-1}$ total radiation) without a levelling off of photosynthesis rate.

The photosynthesis rates of the Aegilops species rose with the incident radiation level up to about $450 \text{ cal cm}^{-2} \text{ day}^{-1}$ but from that point there was no further increase.

The tetraploid Triticum species reached a maximum photosynthesis rate at a lower level of radiation, about $300 \text{ cal cm}^{-2} \text{ day}^{-1}$ and maintained the maximum level up to $600 \text{ cal cm}^{-2} \text{ day}^{-1}$ after which the rate dropped off rapidly.

In the hexaploid species, the early (spring) lines and the late (winter) lines had different responses. In both types photosynthesis rose with light intensity up to a moderate level and then tended to fall as higher levels of radiation were reached. The winter lines had a lower maximum rate of photosynthesis, reached this maximum at a lower level of radiation and the rate fell off more rapidly with increased radiation than in the spring types. Similar variation was found to exist in clones of Mimulus cardinalis collected from six different altitudes (Milner and Hiesey, 1964). When these were grown under similar conditions they responded differently to the temperature and light intensity under which the photosynthesis rate was measured. Clones from high altitudes showed higher rates of photosynthesis and

higher saturating light intensities than those from low altitudes. Eagles and Treharne (1969) working with Norwegian and Portuguese strains of Dactylis glomerata, found that the Portuguese strain increased its rate of photosynthesis per unit chlorophyll with increase in the light intensity under which it was grown, whereas there was little change with the Norwegian line up to 240 w m^{-2} ($240,000 \text{ ergs cm}^{-2} \text{ sec}^{-1}$). The authors suggested that the Norwegian line was dropping in rate at the highest value but their evidence was inconclusive. A marked response to light intensity during growth was also noted by Woledge (1971) with Festuca arundinacea, both in terms of photosynthesis per unit area and per unit dry weight.

The experiment carried out under artificial light (Experiment 5) demonstrated that (at least for the 4 lines used) the adaptation observed was an adaptation to light intensity per se and not to any factor correlating with light intensity in the glasshouse situation.

Clearly much has still to be learnt of the basis of the observed variations in photosynthetic rate. The rates of photosynthesis in the diploid Triticum species under high light seems unlikely to have been associated with the development of the C-4 acid pathway of CO_2 fixation (Hatch and Slack, 1970) associated with high photosynthetic rates in many sub-tropical species. This was unlikely in view of the observation of leaf anatomy by Khan and Tsunoda (1970d) and by the author during the preparation of material in Experiment 6. No "bundle sheath" has been found in any of the Triticum species. In addition there is evidence for high photorespiration rates in all wheat material, a characteristic which has, so far, always been associated with the Calvin cycle pathway of photosynthesis. Dvorak and Natr (1971) have reported high CO_2 compensation rates for a variety of wheat

material, and in the present experiments it was shown that the inhibiting effect of oxygen on net photosynthesis was just as great in the diploid as in the hexaploid species.

Stomatal resistance to CO₂ diffusion could well be one factor contributing to differences in photosynthetic rates between leaves at various positions or developed in different light intensities. Stomatal frequency has been shown to increase with leaf number in Hordeum distichum L., but aperture length and width decreased at the same time (Pazourek, 1969).

Stomatal frequency in Iris hollandica (Pazourek, 1970) and two legumes (Cooper and Qualls, 1967) was found to increase with the light intensity under which growth took place, and furthermore the greatest changes took place in those leaves which showed the highest stomatal numbers for the plant.

Were this finding to apply to wheat species the greatest differences in stomatal frequency would be found in the flag leaves, and the greatest changes with light intensity would be with the leaves of the diploid Triticum species. This conclusion was not borne out by the findings of Experiment 5, where the change in stomatal frequency for the two diploid lines between light intensity treatments was not consistently greater than for the hexaploid lines.

A weak correlation ($r = .52$) was found between stomatal frequency and flag leaf photosynthesis rate for the pooled data of Experiments 3 and 4 (Figure 5 : 2), but this was associated with stronger negative correlations between leaf area and stomatal frequency ($r = .81$) and photosynthesis rate and leaf area ($r = .64$). There is, therefore, no evidence that stomatal frequency is a prime determinant of photosynthesis rate.

It was not within the scope of the thesis to determine

whether the control of photosynthesis in response to genotype leaf position, or light pretreatment was mainly controlled by variation in stomatal resistance (r_s) or in the internal resistance (r_m) of the leaf (see El Sharkawy and Hesketh, 1965). The results of other work would suggest that variation is most likely to occur in the internal resistance and a number of anatomical features likely to be associated with r_m were studied.

Wilson and Cooper (1967) found a negative correlation between mesophyll cross-sectional area and apparent photosynthesis rate. A similar correlation was found in the present work with wheat (Figure 7 : 2). It is usually thought that the higher photosynthesis rates associated with small mesophyll cells result from a higher surface to volume ratio which allows CO_2 to diffuse more rapidly into the cells. However Chonan (1965) found with the mesophyll of hexaploid wheats that larger cells had more protuberances and that the ratio of cell surface to cell volume increased with increase in the number of protuberances per cell. It was noted in the examination of the separated mesophyll cells in the present work that the large cells of the tetraploid and hexaploid lines had more protuberances than the smaller cells of the diploids and that it seems likely that the relation found by Chonan for hexaploid wheats holds for wheats in general. It is therefore not at all clear what mechanism operates to produce the negative correlation between net photosynthesis and mesophyll cell size.

Cooper and Qualls (1967) report that sun leaves were thicker and had higher SLW than shade leaves and that the cells were bigger, more numerous and more clearly differentiated into palisade and spongy mesophyll layers. The same effect was observed in

T. aestivum by Friend and Pomeroy (1970). Chonan (1967) also observed that sun leaves were thicker in rice, had larger cells with more protuberances and had a greater mesophyll surface area to leaf area ratio.

Leaf thickness for the HL pretreatment was as great as or greater than the LL pretreatment in Experiment 5, but the relation between leaf thickness and photosynthesis rate was not clear (Figure 6 : 4).

Pearce et al (1969) found that variation in SLW accounted for 64% of the variation in net photosynthesis rate in alfalfa and that this conclusion held whether the variation was due to genetic or environmental factors.

In the present work correlations between SLW and photosynthesis rate were not found consistently. Correlations between specific leaf weight (SLW) and photosynthesis rate were obtained for diploid species in Experiments 2 and 5 and for tetraploid species in Experiment 2. Differences in SLW did not account for the differences in photosynthesis rate between genotypes as the polyploid species with low rates of photosynthesis tended to have the highest SLW.

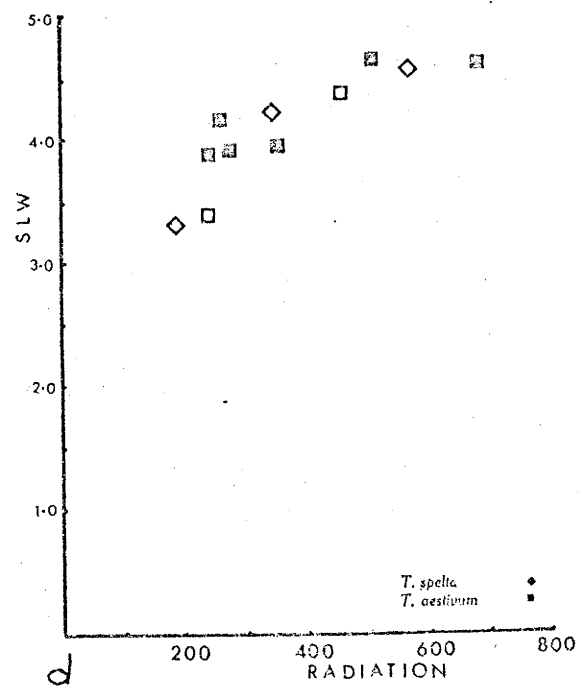
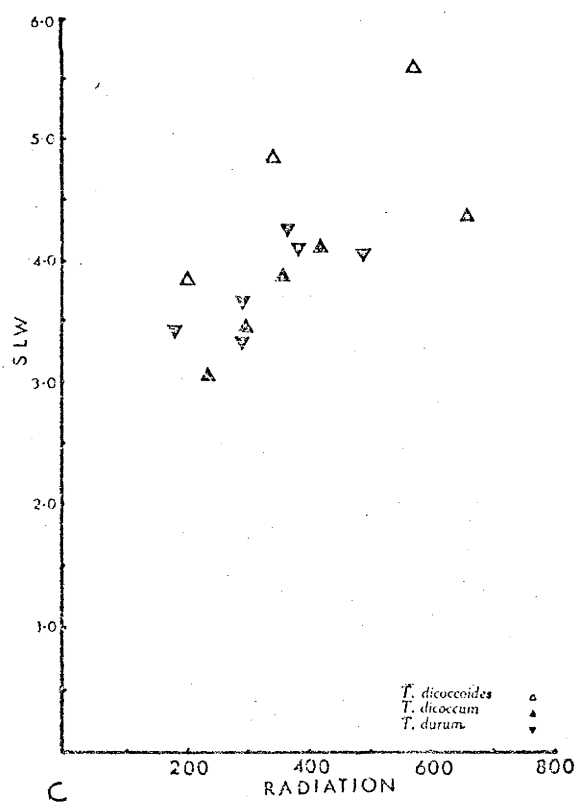
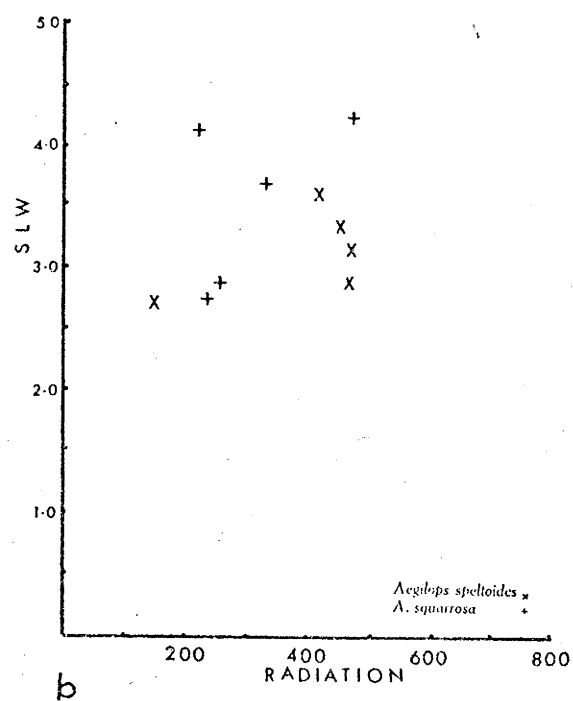
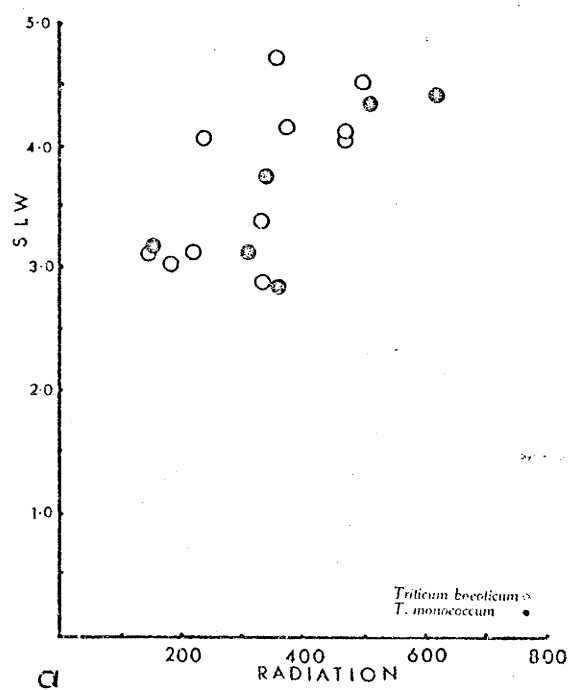
Figure 8 : 2 shows the SLW plotted against irradiation during leaf development for Experiments 2, 3 and 4. (In Experiment 1 SLW was not calculated).

Figure 8 : 2 (Overleaf)

Legend: Relationship between the specific leaf weight (SLW) (mg cm^{-2}) of the flag leaf blade and the mean total daily radiation ($\text{cal cm}^{-2} \text{ day}^{-1}$) above the glasshouse for 3 days prior to harvest. Each point is the mean for one line for one experiment.

Data from Experiments 2 to 4 included.

- | | |
|------------------------------------|-----------------------------------|
| a. Diploid <u>Triticum</u> spp. | b. <u>Aegilops</u> spp. |
| c. Tetraploid <u>Triticum</u> spp. | d. Hexaploid <u>Triticum</u> spp. |
| | (Open symbols, winter varieties. |
| | Closed " " spring varieties. |



In all cases there tends to be an increase in SLW with light intensity. The resultant graphs are very different from the corresponding graphs for photosynthesis rates.

In the tetraploid and hexaploid lines, SLW increased with radiation level but there was no decline at the highest radiation levels as was observed for the photosynthesis rates. Neither is there a separation in the responses of the spring and winter hexaploid lines. Increase in SLW may explain the increased rate of photosynthesis with increased radiation at low levels but it does not explain the depression of photosynthesis rates in some species at high light.

The present work shows that primitive diploid species can adapt to high irradiance levels in their photosynthesis rates whereas the modern tetraploid and to a greater extent the hexaploid cultivars are strongly inhibited by high light during development. However, the interaction of changes in leaf area and changes in maximum photosynthetic rate with the radiation level during development, results in the photosynthetic rate per total flag leaf having a different pattern of adaptation to radiation than the photosynthetic rate per unit area.

Figure 8 : 3 shows the total flag leaf photosynthesis ($\text{mg CO}_2 \text{ flag leaf}^{-1} \text{ hr}^{-1}$) plotted against the mean daily irradiance for three days before measurement. From this graph we can see the effect that radiation level during development has upon the maximum level of availability of fixed carbon from the flag leaf. It is clear that selection has resulted in a plant with a flag leaf which has a greater potential at low levels. Moreover there appears to be a different degree of adaptation between the winter hexaploid lines and the spring hexaploid lines.

Figure 8 : 3 (Overleaf)

Legend: The relationship between the total flag leaf photosynthesis ($\text{mg CO}_2 \text{ flag hr}^{-1}$) and the mean total radiation ($\text{cal cm}^{-2} \text{ day}^{-1}$) received for 3 days before measurement. Each point is the mean for one experiment. Experiments 1, 2, 3 and 4 included.

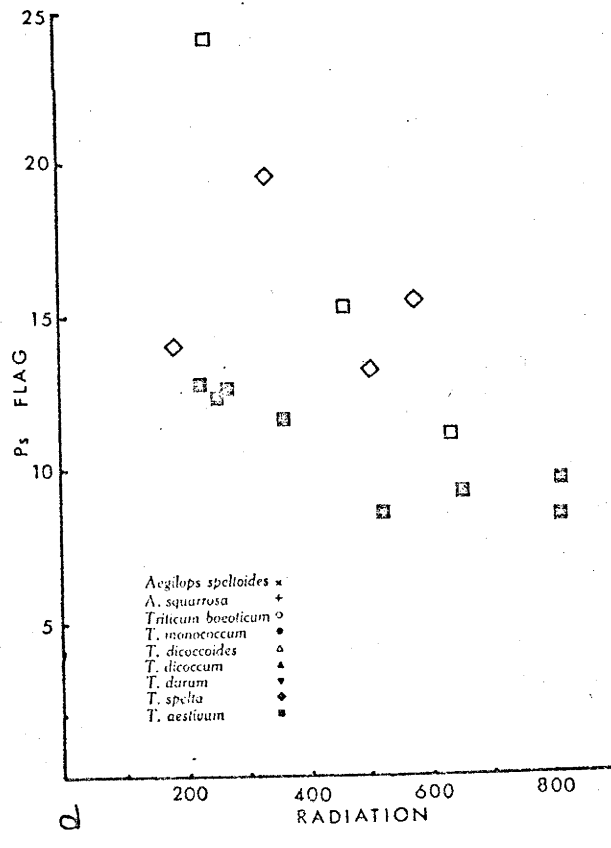
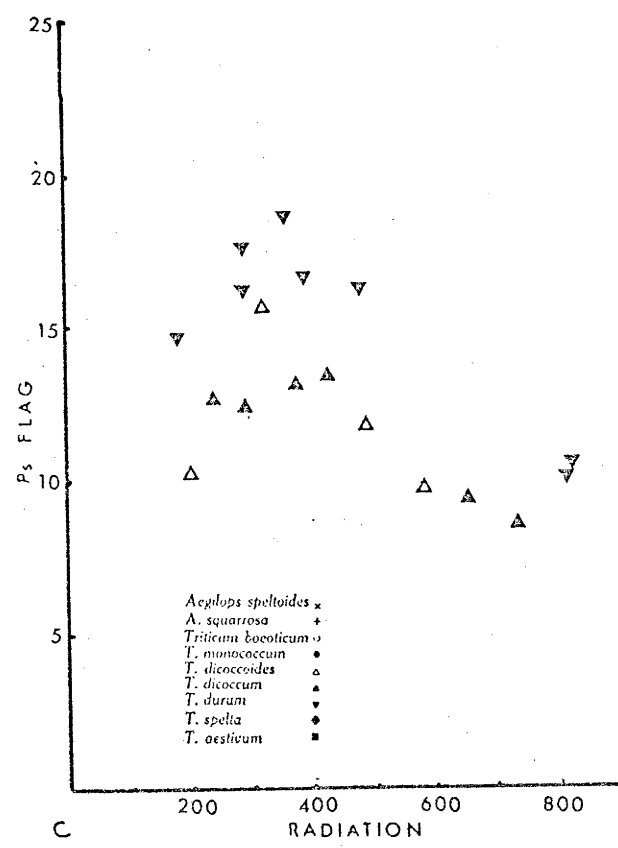
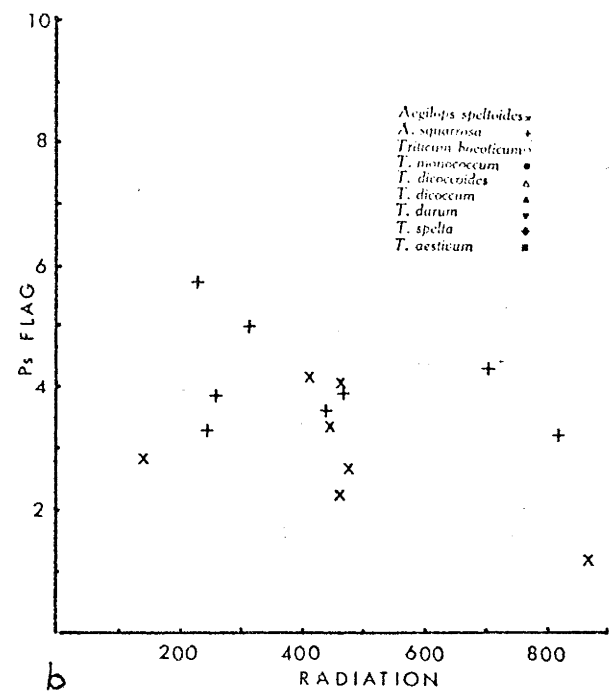
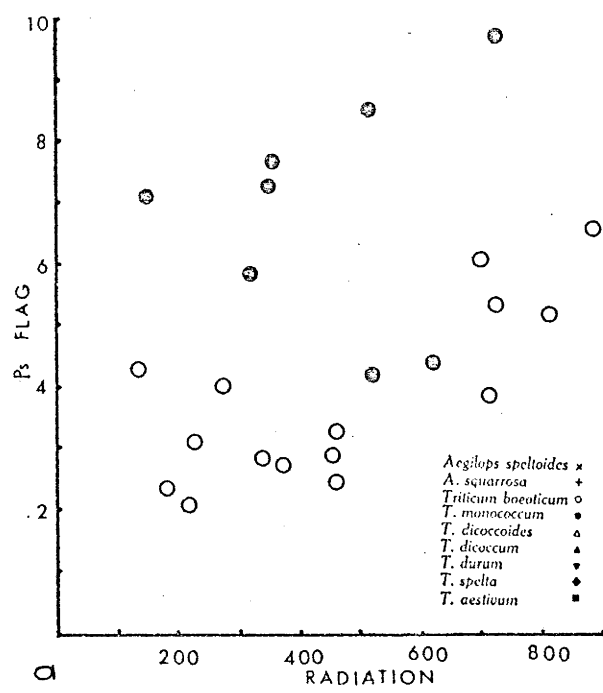
a. Diploid Triticum spp.

b. Aegilops spp.

c. Tetraploid Triticum spp.

d. Hexaploid Triticum spp.

(Open symbols, winter varieties.
Closed " , spring varieties).



Distribution and Transport

The total dry weight at maturity of some of the wild progenitors was comparable to that of the modern wheats, but its distribution was very different (Experiment 1). Evolution appears to have been accompanied by increased apical dominance following inflorescence initiation. Not only is the plant substance concentrated in fewer tillers, but more of that within each tiller is concentrated in the grain. The small, slow-growing ears of A. speltoides were largely self-supporting for photosynthate, as indicated by the small proportion of ^{14}C -labelled photosynthate translocated to the ears from the flag leaf (Experiment 1). In the other diploids this proportion was higher, particularly in T. boeoticum with its extremely small flag leaves. The proportion rose to still higher levels in the tetraploid and hexaploid wheats, whose ears also drew to a greater extent on assimilates from the leaves below the flag leaf to support their much higher rates of grain growth. Thus, the extent of import by the ears has increased progressively with evolution, and this has been accompanied by a proportional increase in the cross-sectional area of phloem tissue at the top of the stem (Evans et al 1970). Similarly, the fall in stem weight from its maximum until maturity of the ear has tended to increase with increase in ploidy and with selection for cultivation (Table 3 : 1). The great increase in the rate and extent of grain growth with evolution in wheat has thus probably involved a greater mobilization of photosynthate from the stems and leaves, and an increase in the capacity of the culm phloem to transport it to the ear.

Accumulation

There is evidence in the literature (Evers, 1970) and from the examination of the grain sections in the present work (Experiment 6), that the centre of the lobes of large grains is more

tightly packed with starch grains than the sub-aleurone layers. This could imply a change of density of the grain with increase in grain size. However, it can be seen from Figure 7 : 3 that there was no change in density. The increase in grain weight with evolution has been entirely due to an increase in grain volume.

The increase in grain volume was not related to increase in endosperm cell size (Figure 7 : 4) although some increase in cell size with evolution has been apparent. It is clear that much of the increase in grain volume was due to an increase in cell number but it is not clear how the interaction of cell size increases and cell number increases has developed.

The percentage nitrogen of the grain has decreased with evolution (Figure 7 : 6). Evers (1970) noted that the protein concentration in the sub-aleurone endosperm was higher than in the inner layers and an examination of the grain sections stained with Ponceau 2R supported this finding. It follows that larger grains with a smaller proportion of sub-aleurone endosperm would have lower protein concentrations.

Conclusions

The evidence suggests that increase in grain and leaf size, and in the proportion of dry weight mobilised to the grain, have been the dominant physiological changes in the evolution of yield in wheat. There was no evidence to show that the parallel increase in grain and leaf size with evolution was due to a general increase in cell size, although increase in cell size was important in some instances.

The changes that have taken place in photosynthetic rate per unit area with evolution are quite complex. When the plants were grown under the high light conditions of Experiment 1 it was evident that photosynthesis rate had decreased with evolution. Later

experiments showed that the way in which photosynthesis rate changed in response to the light intensity under which the plants were grown had also changed with evolution.

In terms of the physiological processes I have examined, the biggest evolutionary advance was in the step from diploid to tetraploid genomes. The wild tetraploid lines I used were both of the robust Palestine race which Harlan and Zohary (1966) consider to be the likely progenitor of most modern wheats. In grain and leaf size and other features T. dicoccoides was much more advanced than T. monococcum. Moreover the diploid species did not adapt at all well to low light conditions and it seems doubtful if these plants could be grown successfully in low light areas such as northern Europe.

My results support Bell's (1965) comment that cultivated wheat would not have progressed far at the diploid level. The greatest value of T. monococcum presumably lies in its resistance to frost, drought, rust, poor soils, and the depredations of birds (Percival, 1921). On the other hand, grain yield per ear and many other features of the durum wheats were comparable to those of the hexaploids. Thus, in terms of the physiological processes determining productivity, the addition of the D genome may have had little effect, as Shebeski (1958) has already suggested, and its main contribution may have been to baking quality, the spectrum of disease resistance, and, as Zohary, Harlan, and Vardi (1969) suggest, to the adaptive range of wheat.

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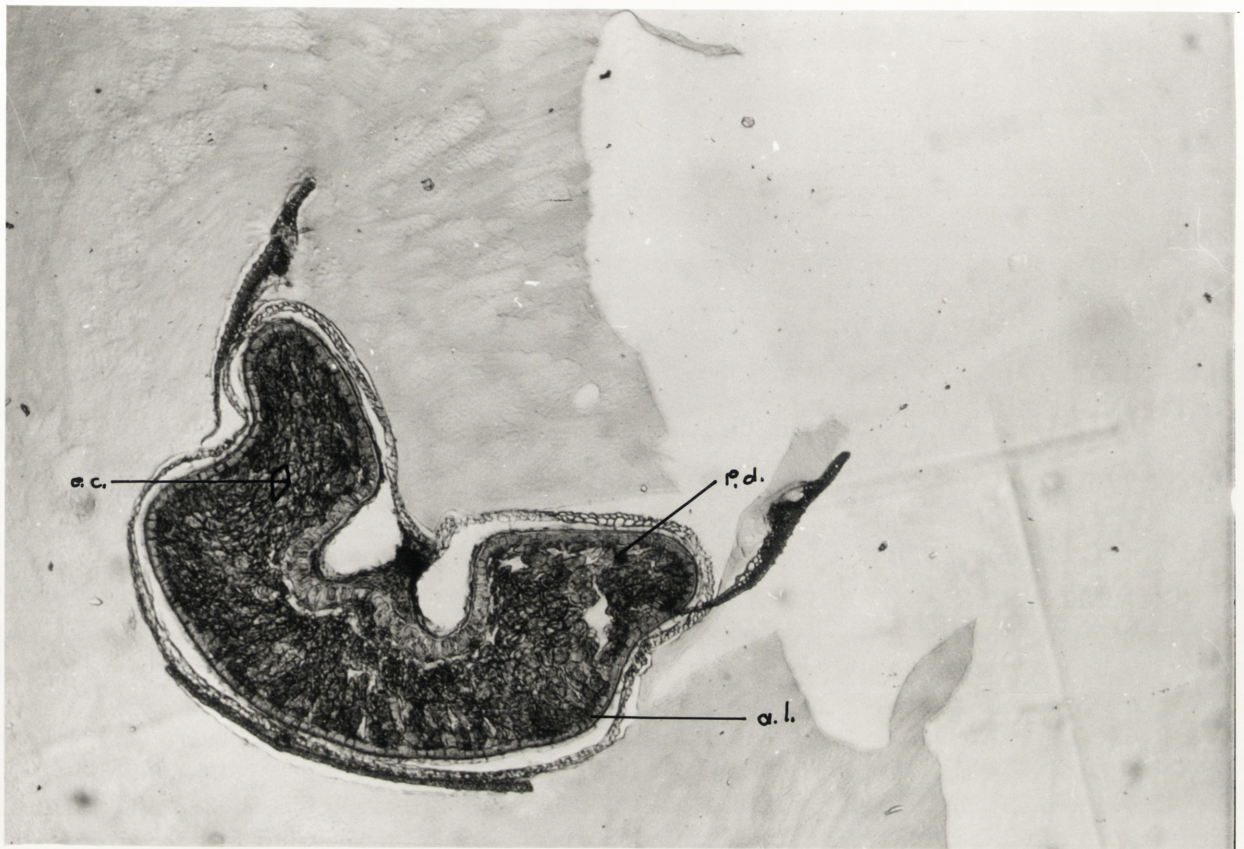
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APPENDIX 1

Plates 1 to 9.

Cross sections of grains, 10 u thick, stained with Ponceau 2R stain.

a.l. aleurone layer
p.b. protein body
e.c. endosperm cell.



1. Ae. speltoides

1 mm.



2. Ae. squarrosa

Diploid



3. T. boeoticum

1 mm.



4. T. monococcum

Tetraploid



5. T. dicoccoides

1 mm.



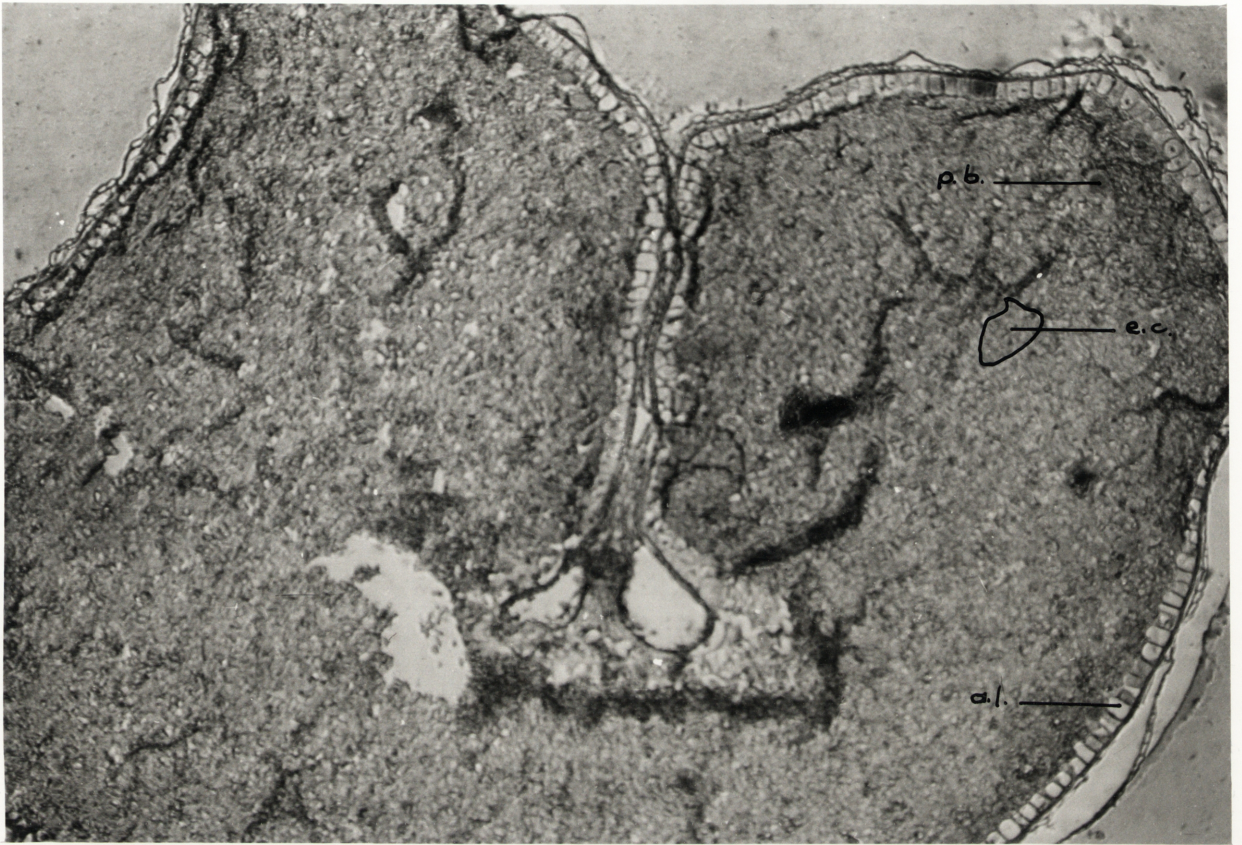
6. T. dicoccum

Tetraploid

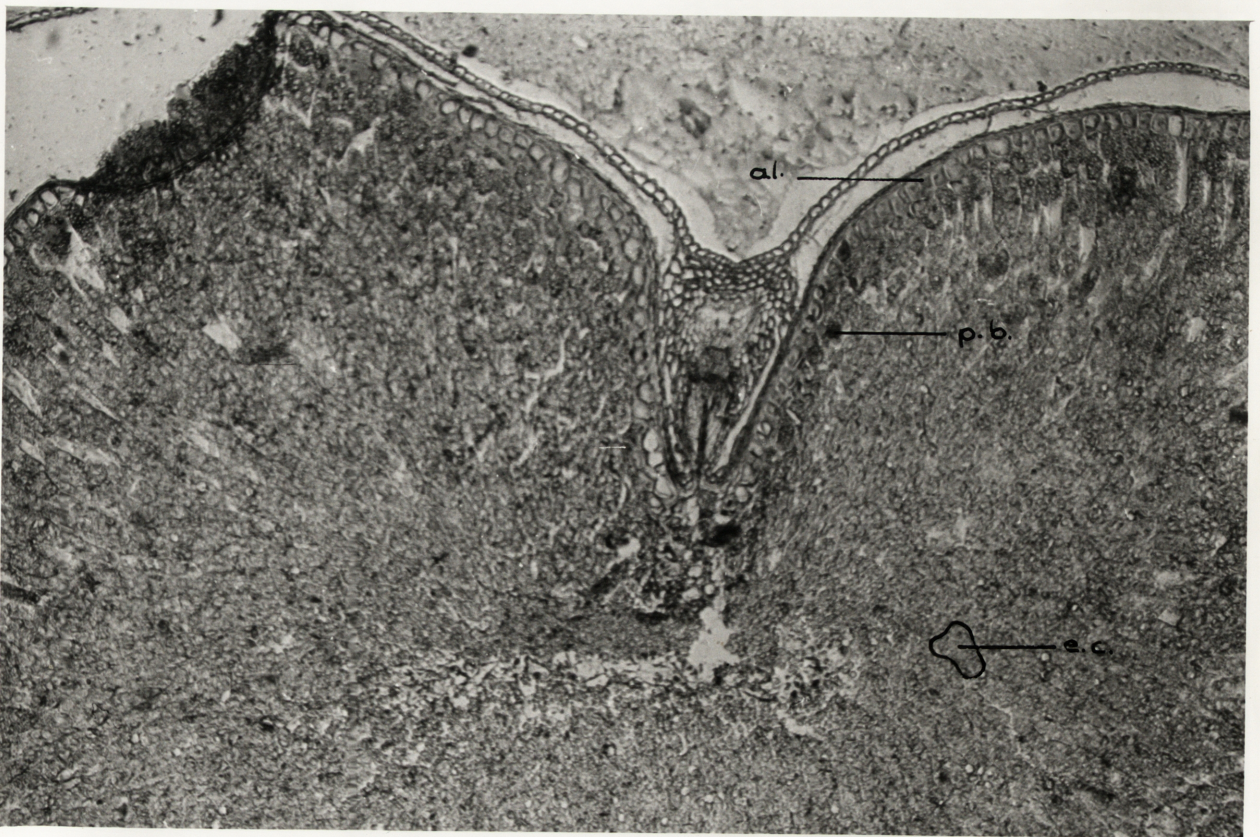
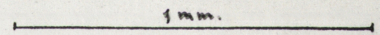


1 mm.

7. T. durum



8. T. spelta



9. T. aestivum

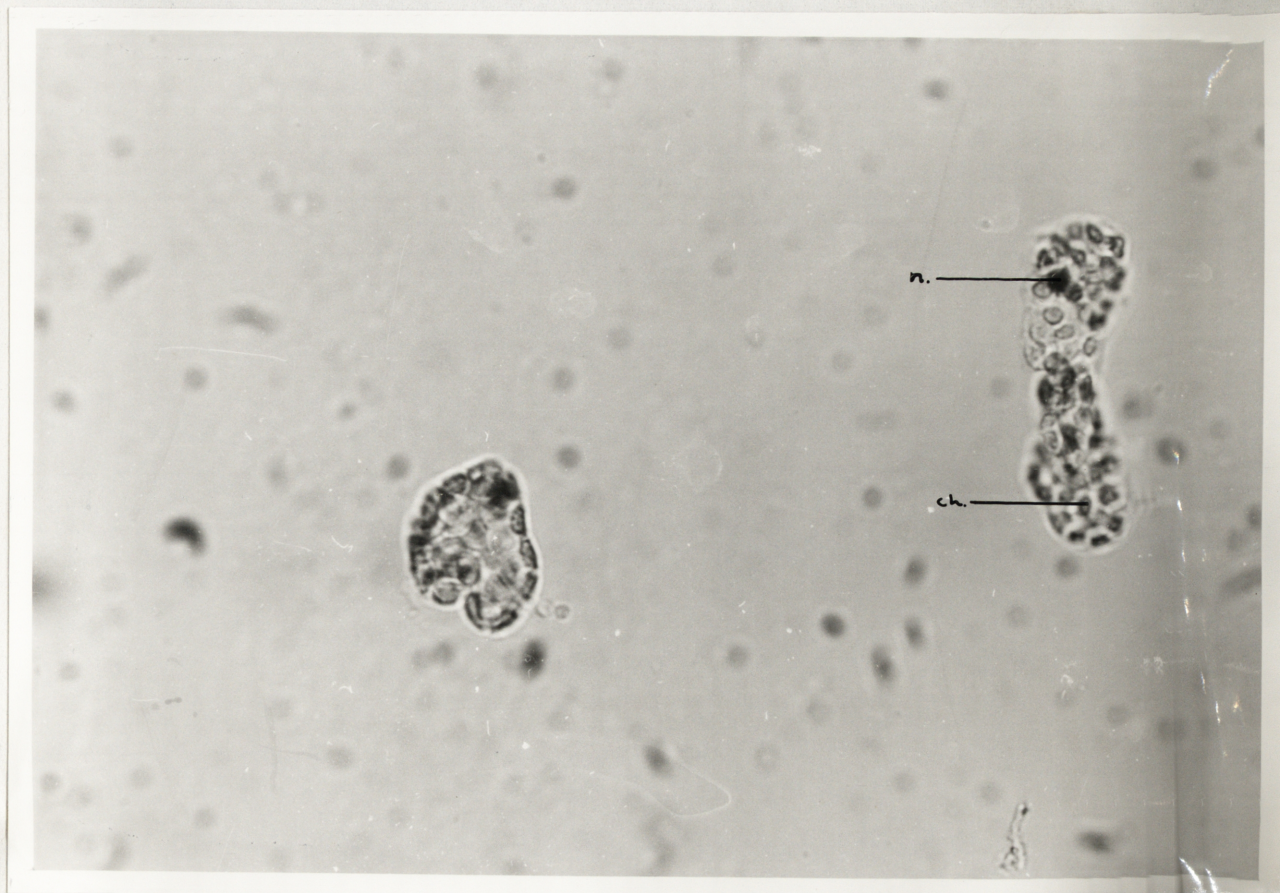
Plates 10 to 18.

Suspended mesophyll cells stained with Feulgens stain.

n. nucleus.
ch. chloroplast.

10. Ae. speltoides

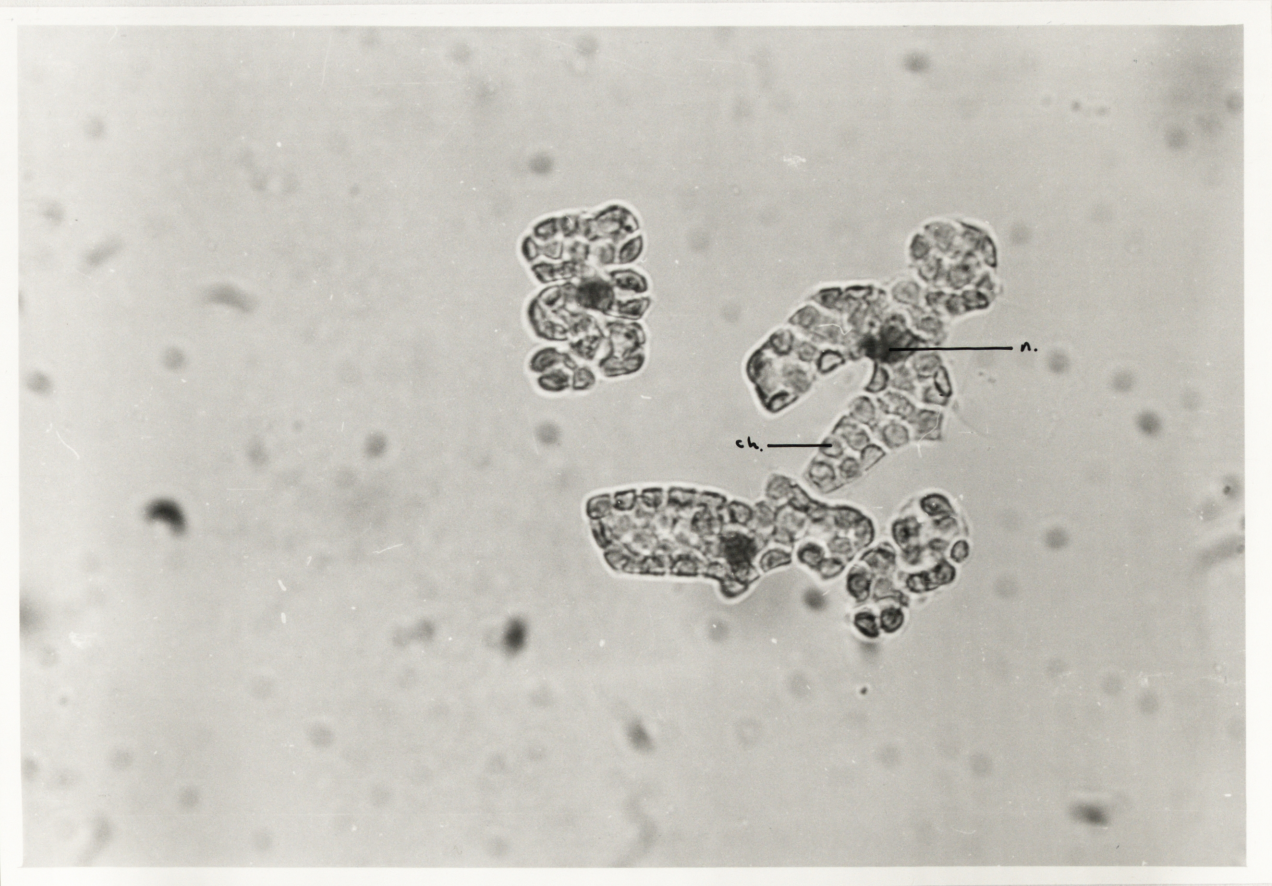
50 μ

11. Ae. squarrosa



12. T. boeoticum

50 μ

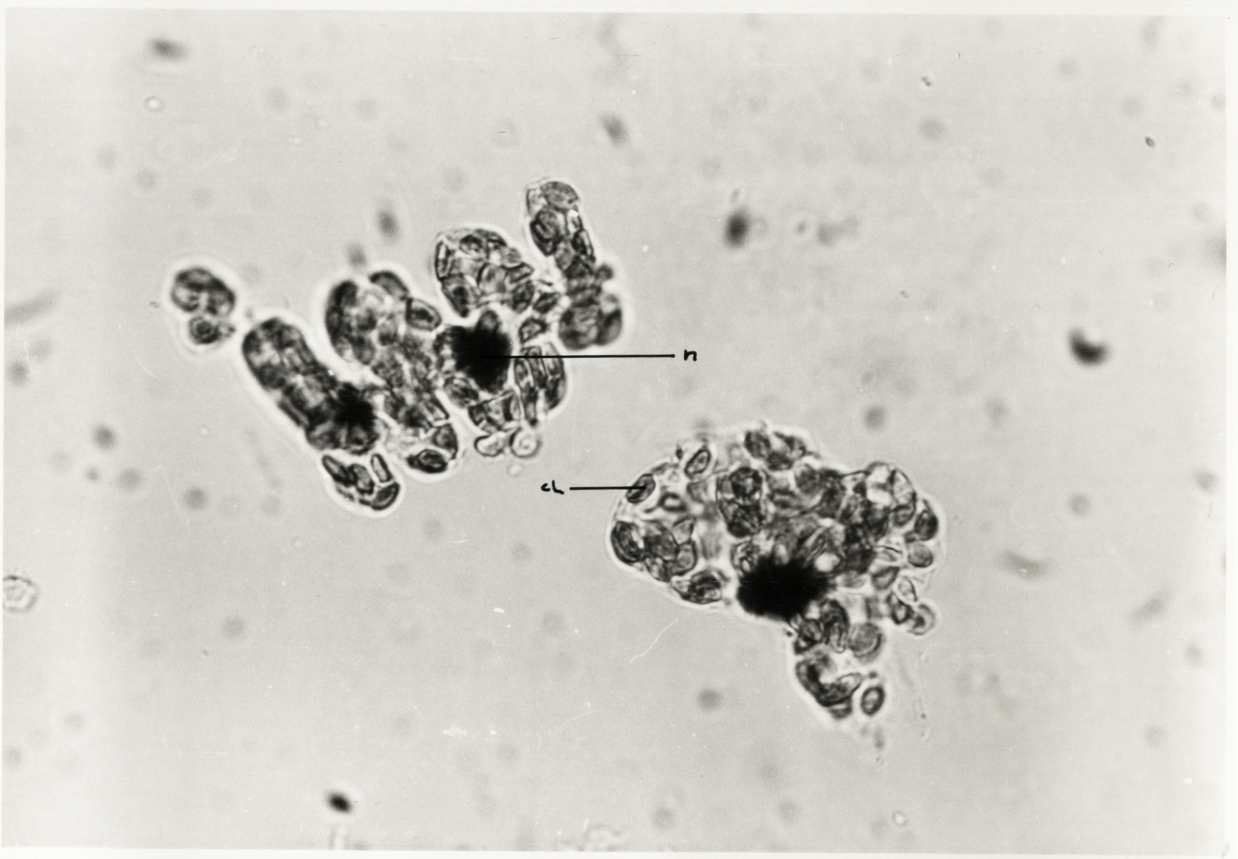


13. T. monococcum



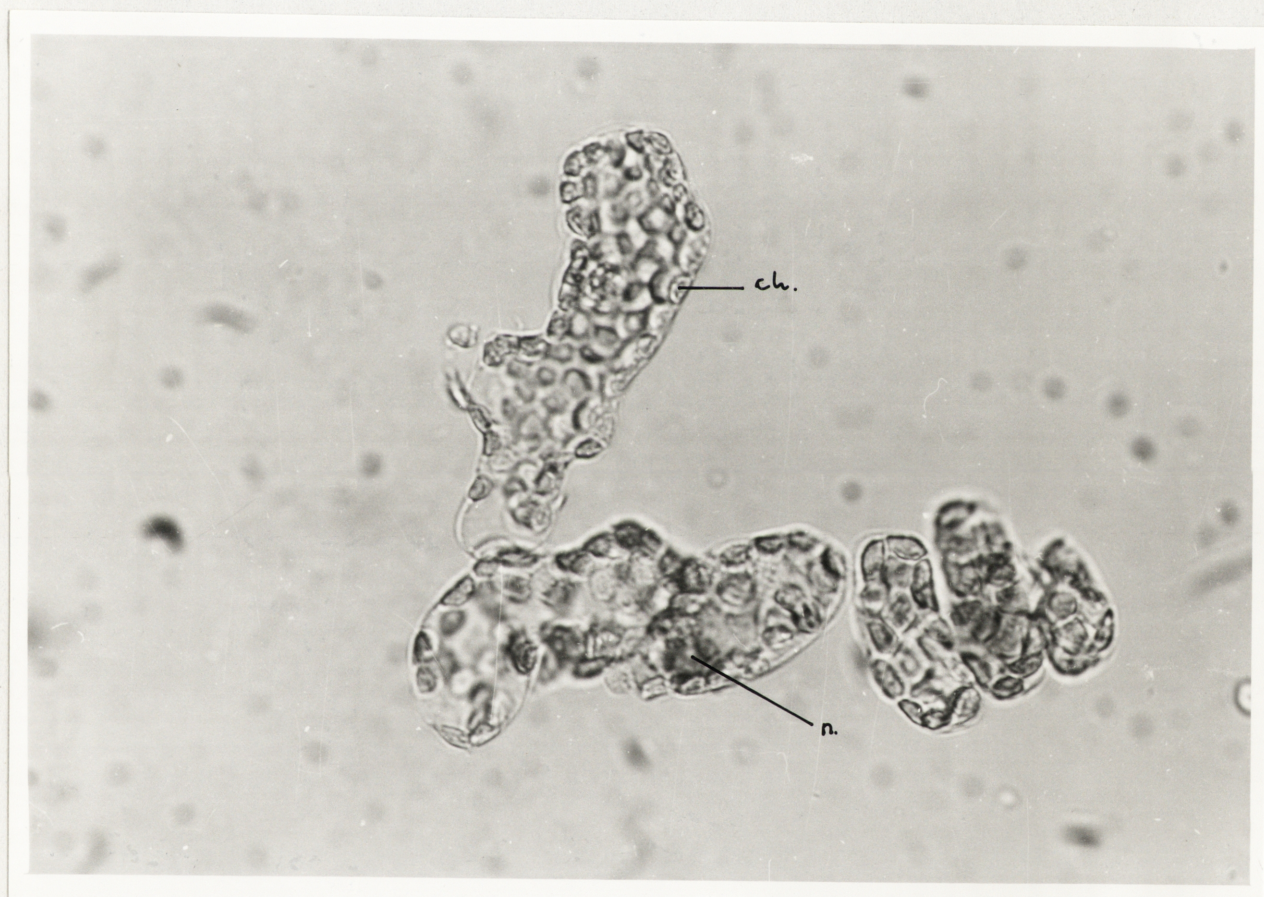
14. T. dicoccoides

50 μ



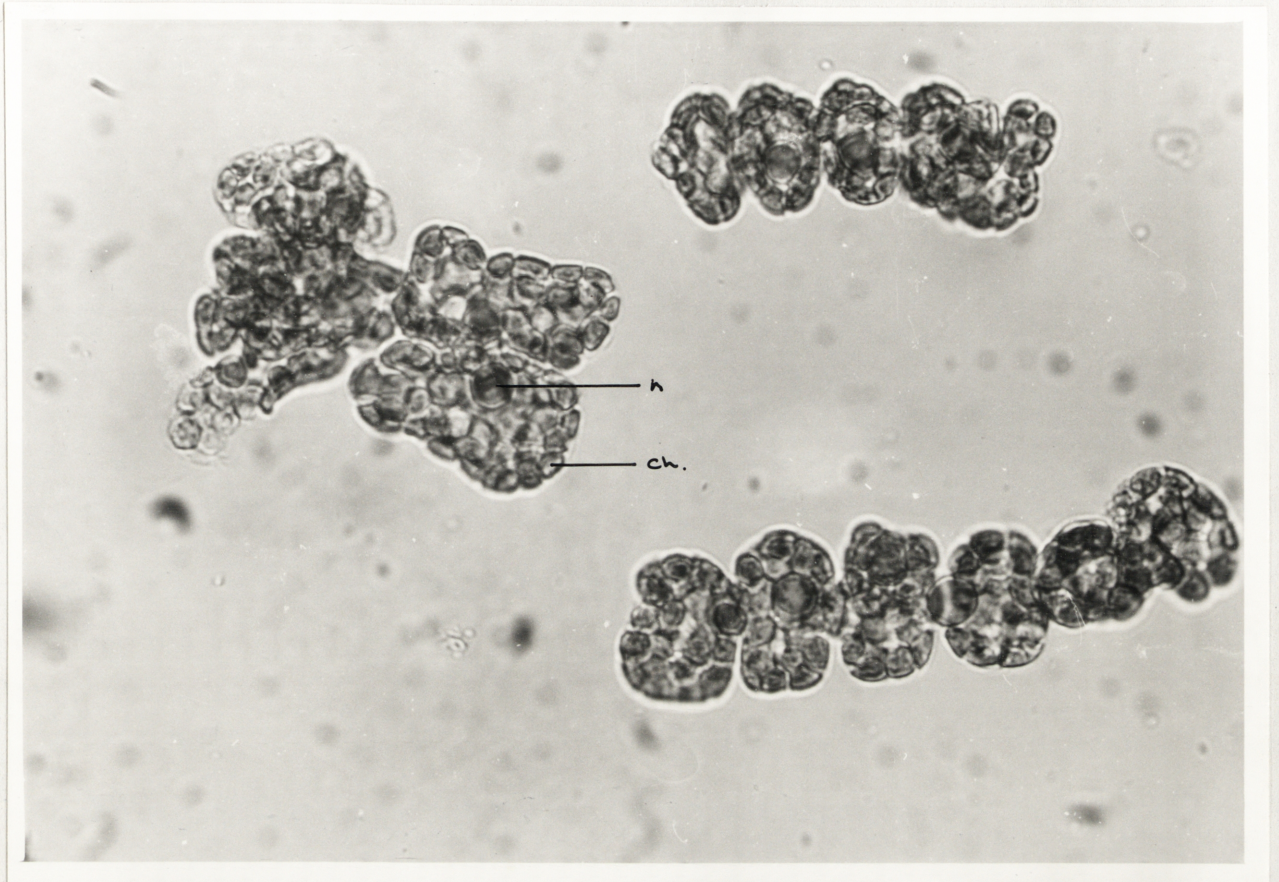
15. T. dicoccum

Tetraploid



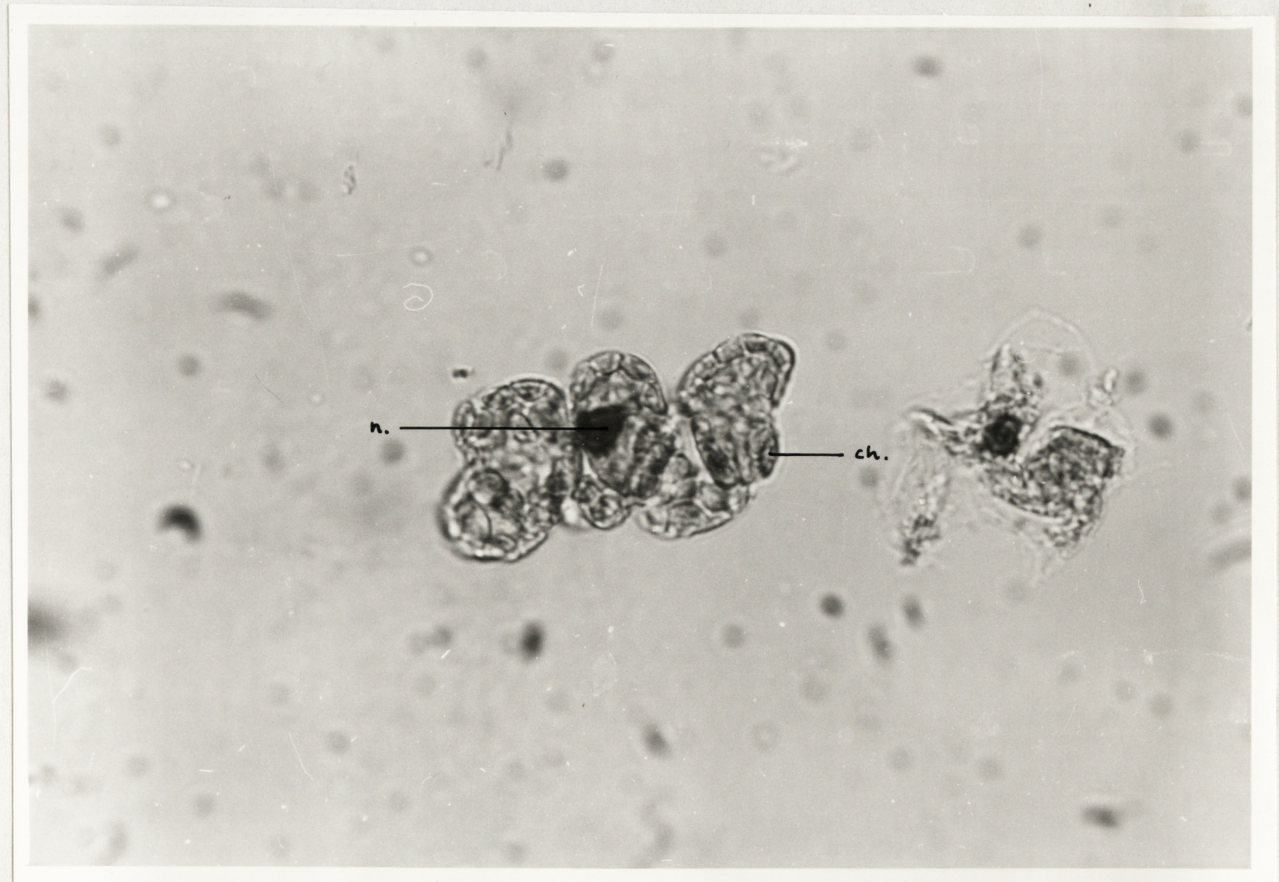
50 μ

16. T. durum



17. T. spelta

50 μ



18. T. aestivum